Volume XXXVII March, 1934 Number 3

SOIL SCIENCE

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JACOB G. LIPMAN

Associate Editor
HERMINIE BROEDEL KITCHEN

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Published Monthly by THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MARYLAND, U. S. A.

Made in United States of America

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THE NATURE OF SLICK SOIL IN SOUTHERN IDAHO[1]

PHILIP ISAAK

Idaho Agricultural Experiment Station

Received for publication July 24, 1933

In the southwestern part of Idaho there are spotted areas in the soil that hardly maintain plant growth. Kelley and Brown (17) and Peterson (22) have called attention to these areas. They are commonly known as "slick spots" from the fact that they will not let water pass through and, when wet, give a shiny appearance in contrast to the surrounding soils, which give a normal appearance of a soil when moist.

The terms "slick" and "normal" soil will be used throughout this paper to designate this abnormal soil and the normal soil surrounding it.

Ordinarily these slick spots are from a few to several hundred square yards in size and are promiscuously scattered in a field. The location of these spots has no particular reference to the topography of the land, and the only way they can be recognized is by the land being void of any vegetation, which is mostly sagebrush. In a cultivated field they may be recognized by the poor physical condition of the soil when dry, or the aforenamed shiny appearance when wet, and by deficient plant growth if crops have been planted.

Soil profiles were examined on a number of these spots and in the adjacent normal soils. This study brought out one thing: Below both the slick, and normal soils there is a rather heavily impregnated calcareous layer which occurs at varying depths, ranging from 5 to 15 inches below the surface. The calcareous layer may vary in depth, but normally extends several feet below the surface. Much of this layer is underlaid by a caliche layer; very commonly there is in addition a layer of coarse gravel, the shape of which shows that the soil is water deposited.

PURPOSE OF THE EXPERIMENT

Since the slick spots at times comprise an area from 10 to 40 per cent of the total in a field, their reclamation becomes of great economic importance, and ways and means have been sought to improve them with the lowest expenditure. In order to find some of the fundamental differences that exist between the slick and the normal soils, and to see how different admixtures of these soils may affect each other, a set of samples was made up to serve as a basis for study of the plant relationship to the physicochemical make-up of these soils.

[1] Published with the approval of the director as Research Paper No. 103 of the Idaho Agricultural Experiment Station.

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PREPARATION OF SAMPLES

A set of 10 bulk samples was taken at the Caldwell Substation in the fall of 1930. They were taken at a depth of 7 inches in the desert, and each slick spot sample had a corresponding normal sample, taken as close as possible to the slick spot but in a place where the vegetation was normal. The samples taken were large enough to permit carrying on of greenhouse work and were shipped to the experiment station laboratory at Moscow, where all the work was done. A series of 11 samples, numbers 10234 to 10244, was then prepared, using the proportions of slick to normal soil ranging from 10 to 0 in the first to 0 to 10 in the last.

The main portion of each was put in 4-gallon jars, 40 pounds to a pot, for greenhouse studies, and subsamples of each were taken for studies in the laboratory.

TABLE 1

Chemical analyses on slick and normal soils from Caldwell Substation

NUMBER	DESCRIPTION		SiO_2	Al_2O_3	Fe_2O_3	P_2O_5	CaO	MgO	K_2O	Na_2O	LOSS IN IGNITION	N
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
18	Surface	Slick	65.62	14.70	4.49	0.11	3.82	1.47	2.42	2.31	1.67	0.06
20	Surface	Normal	67.97	14.05	4.52	0.25	3.45	2.33	2.63	2.51	2.20	0.07
22	Surface	Slick	63.28	14.78	3.17	0.23	3.90	3.64	2.48	1.39	3.88	0.05
24	Surface	Normal	69.70	15.34	3.48	0.18	2.91	1.74	2.37	2.36	3.89	0.08
41	Surface	Slick	63.10	15.66	5.07	0.15	2.04	1.70	1.62	2.55	4.52	0.11
42	Surface	Normal	62.40	15.43	5.90	0.18	2.59	1.34	2.56	2.96	5.25	0.01
1	Surface	Slick	67.22	16.15	4.65	0.12	2.99	1.33	2.06	2.81	3.37	0.04
2	Surface	Normal	70.15	15.79	4.31	0.11	1.79	1.11	2.22	2.54	3.03	0.06

It was felt that this was a safe procedure because chemical analyses obtained from the work of Peterson (22) on samples from the same locality (samples 18 to 42 in table 1) and analyses of samples 1 and 2 made in connection with some other work (unpublished data) show that the chemical composition of these soils varied very little. The pH determinations, too, made on the original samples at the time they were taken, did not show great differences within each group.

RESULTS OF GREENHOUSE STUDIES

The soil samples in pots were planted to corn, 10 seeds to each pot, and after the plants were about 1 inch high they were thinned down to three plants in a pot. Each soil was run in triplicate. Records were kept of all moisture added. In the pots where the soil was predominantly slick the moisture was taken up very slowly, and when dry the surface was full of cracks. The size of the cracks decreased as the proportion of the normal soil increased over that of the slick soil. There is no doubt that the difference in the cracks is due to

the different amounts of colloidal material present in each soil; mechanical analysis of these soils, as will be shown later, proved that to be the case.

Additional water was added to the pots only after the plants commenced to wilt. This was done for the purpose of using only the minimum amount of water to produce the maximum plant growth. The pots with the slick soil or mostly slick soil usually required water at least a week ahead of the pots with the normal soil.

After the corn had tasseled, it was cut even with the ground and oven dried. The amount of water actually used in the process of growing was computed and the amount of water used to produce 1 gm. of dry matter was calculated. The results and also the moisture content of the soils after the completion of the experiment are shown in table 2.

TABLE 2

Data of greenhouse work

SOIL NUMBER	SUM OF THREE POTS		AMOUNT OF H_2O USED TO PRODUCE 1 GM. OF DRY MATTER	MOISTURE CONTENT OF SOIL AT TIME OF HARVEST—AVERAGE OF THREE POTS
	Amount of H_2O used during growth	Weight of dry matter produced		
	cc.	*gm.*	*cc.*	*per cent*
10234*	9,214	4.42	2,085	10.7
10235	13,400	6.78	1,990	11.3
10236	13,317	7.23	1,845	11.4
10237	13,598	13.11	1,035	10.8
10238	14,082	18.92	745	9.7
10239	14,380	21.06	683	8.7
10240	14,695	24.58	597	8.3
10241	15,151	19.46	775	6.9
10242	15,330	20.48	747	6.5
10243	16,063	20.12	798	5.2
10244	15,971	22.48	709	5.3

* Run in duplicate only.

There is a distinct gradation in the amount of water used to produce 1 gm. of dry matter for the different soils. Samples 10239 and 10240, 50 and 40 per cent slick soils, respectively, show a small difference from the regular trend. This probably was due to a slightly lighter packing of the soil when planted, which resulted in the plants getting a better start at the beginning of the experiment.

Two factors are responsible for the uneven utilization of water: first, there is a higher evaporation of water from the surface of the pot in the slick soil, and secondly, there is an actual difference in yield in favor of the normal soils. These two points will be discussed later.

RESULTS OF LABORATORY STUDIES

High alkalinity of soils is considered one possible cause of deficient plant growth in irrigated sections. That this is not the case with slick soils is shown

by the fact that all the soils used in this work had comparatively low pH values and contained relatively small amounts of water-soluble salts.

The pH determinations are reported in the last column of table 3; the hydrogen electrode being used for the determinations. Water-soluble salts were

TABLE 3

Exchangeable bases and pH in soils

SOIL NUMBER	Al	Ca	Mg	Na	WATER-SOLUBLE Na M.E. PER 100 GM. OF SOIL	RATIO OF EXCHANGEABLE Ca:Na	pH OF SOIL
	m.e. of exchangeable bases per 100 gm. of soil						
10234	0.89	9.83	3.14	5.75	6.22	1.71	7.99
10235	0.50	9.53	3.09	4.95	5.73	1.93	7.89
10236	0.50	9.41	3.01	4.71	5.01	2.00	7.93
10237	0.38	9.27	2.75	3.77	4.58	2.46	7.86
10238	0.38	8.92	2.54	3.52	4.00	2.54	7.69
10239	0.38	8.34	2.46	3.08	3.30	2.71	7.52
10240	0.30	8.34	2.30	2.09	3.10	3.99	7.52
10241	0.30	7.50	2.12	1.52	2.44	4.93	7.53
10242	0.21	7.62	1.94	1.20	1.66	6.35	7.52
10243	0.21	7.50	1.76	0.46	1.27	16.30	7.53
10244	0.21	7.26	1.62	0.15	0.48	48.40	7.30

TABLE 4

Mechanical analysis of soils

SOIL NUMBER	DIAMETER OF PARTICLES IN MILLIMETERS							
	2.0–1.0	1.0–0.5	0.5–0.25	0.25–0.10	0.10–0.05	0.05–0.005	0.005–0.002	Colloid <0.002
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
10234	0.49	0.52	1.59	6.57	12.16	50.01	6.22	23.40
10235	0.29	0.86	1.78	6.95	13.75	49.50	6.01	21.80
10236	0.42	1.08	1.88	7.08	14.87	49.25	5.83	20.50
10237	0.44	1.13	2.15	7.25	15.92	50.30	5.07	18.68
10238	0.37	1.18	2.24	8.12	16.07	51.10	5.38	16.65
10239	0.47	1.00	2.20	7.98	17.00	50.70	5.13	15.95
10240	0.43	1.25	2.26	8.17	18.62	50.50	4.90	14.09
10241	0.45	1.15	2.46	9.13	19.28	50.57	4.86	12.96
10242	0.38	1.27	2.58	9.18	19.81	50.20	5.22	11.32
10243	0.39	1.38	2.53	9.96	22.03	48.80	5.02	10.11
10244	0.44	1.51	2.76	9.94	22.35	50.70	4.75	8.57

run in connection with the determination of the exchangeable bases, and only soluble Na was found; the amounts are given in table 3.

It is felt that the low hydrogen-ion concentration is due to a low water-soluble salt content. Breazeale and McGeorge (5) and Kelley and Brown (17) mention a low water-soluble salt content of slick soils. They do not indicate the pH of these soils, however.

It has been recognized by many soil investigators [Anderson (1), Gedroiz (9, 11, 12), Cummins and Kelley (7), Hissink (14), Kelley and Brown (17)] that the exchangeable Ca and Na have a profound influence on the properties of the soil. Determinations of exchangeable bases of the soils studied were first made according to the HCl method of Gedroiz (10) as used by Puri (24), but the fact that this method gives large quantities of aluminum and iron in the extract makes it a very doubtful method. For that reason the ammonium acetate method of Schollenberger (25) was finally adopted. The results are given in table 3.

The ammonium acetate extract showed only traces of K in the first three samples. No exchangeable K was found in the remaining soil samples.

As a further study the mechanical analysis of these soils was made. The determination was made according to the method of Olmstead, Alexander, and Middleton (21). The results are given in table 4.

TABLE 5

Dispersion factor and dispersion coefficient determinations

SOIL NUMBER	D. F.	COLLOIDAL CLAY	D. C.
	per cent	*per cent*	*per cent*
10234	4.45	23.40	19.00
10235	4.66	21.80	21.35
10236	4.19	20.50	20.50
10237	3.72	18.68	19.90
10238	3.46	16.65	20.80
10239	3.66	15.95	22.90
10240	3.53	14.09	25.05
10241	3.42	12.96	25.40
10242	3.27	11.32	28.85
10243	3.39	10.11	33.40
10244	3.05	8.57	35.70

At the same time, two factors proposed by Puri (23) to determine the physical state of a soil, the dispersion factor (D. F.) and dispersion coefficient (D. C.), were determined. The results are shown in table 5.

DISCUSSION OF RESULTS

It was stated in the beginning of this paper that no actual chemical analyses were made on the samples used in this work, but analyses taken from Peterson's work and chemical analyses made in connection with other work (unpublished data) show only significant differences in the SiO_2 content if samples 41 and 42 are disregarded (table 1). It would be more appropriate to study the silica-alumina ratio in colloidal material for these soils, since Anderson (1), Botkin (4), Bennett (3), and others have pointed out that the soil colloidal material varies apparently with the content of exchangeable bases and with the ratio of $\frac{SiO_2}{Al_2O_3}$. This, however, will be made a separate study in the near

future, together with some other factors involved in the physicochemical state of a slick soil.

Hydrogen-ion determinations (table 3) show a distinct gradation for the soils used, with somewhat higher values for the slick soil. These differences, however, are not large enough to attach any significance to these data, since a pH in a soil as high as 8 is still considered a favorable medium for plant growth.

In a preceding paragraph two reasons were given for a poor production of plant material. In both cases the real cause can be traced back to the poor physical condition of the soil. Losses of water take place because moisture does not penetrate into the soil and on prolonged standing on the surface of the soil is evaporated. In a soil with a poor physical condition the root system of a plant cannot develop very effectively or utilize to the best advantage the nutrients available. Numerous investigators have shown that different cations are responsible for a certain physical condition of a soil. Anderson (1), Baver (2), and Gedroiz (8, 9), have shown that Na-saturated colloidal material is more dispersed than that which is Ca-saturated. Mattson (20) showed that a Na clay swelled more than a Ca-saturated clay, and that the Na increased the charge of a colloid (18, 19). Anderson believes that each cation exerts a characteristic influence in proportion to the quantities present. Baver, however, states that "the properties of the system are not directly proportional to the degree of substitution of any one base for another. This is especially true in the comparison of the effects of the monovalent and divalent ions." Puri (23) showed that soils saturated with different cations, although having about the same clay content, have different deflocculation factors and dispersion coefficients.

Hissink (13, 15), Kelley and Brown (16), and Singh and Nijhawan (26) suggest the ratio of divalent to monovalent ions as particularly important indicators of the properties of the soils. Burgess and Breazeale (6) state, "little or no idea of the degree of dispersion of a soil can be gained from the study of the Ca:Na ratio." Data obtained on our soils show a positive correlation between the Ca:Na ratio and the amount of water necessary to produce a gram of dry weight of crop. Whether the deficient plant growth is due to a direct toxic effect of a high replaceable sodium content, as suggested by Kelley and Brown (16, 17), or due to the unfavorable physical condition of the soil, is difficult to tell. Further work is necessary to clear up this point. Probably both factors have an effect, but it is very likely that the latter is more important.

This last point could be partly cleared up by making a mechanical analysis of a soil to see to what extent the fine material predominates over the coarser fraction in the slick soil as compared with the normal soil. The mechanical analyses of these soils are given in table 4. It was of particular interest to run these analyses because of the work done at this station by Peterson, in which he found no appreciable difference in the mechanical composition of slick and normal soils. According to our data there is an increase in the fraction com-

prising the particle size beginning with the 1.00–0.5 mm. group and down through the 0.1–0.05 mm. groups in the normal soil over that of the slick soil. The fraction for the 0.05–0.005 mm. size remains constant at about 50 per cent, disregarding rather small discrepancies in a few samples. For the two fractions of 0.005–0.002 mm. size and the so-called colloidal fraction for particles smaller than 0.002 mm., the reverse is true, i.e. the larger proportion is found for the slick soil and the per cent of material decreases as the amount of normal soil increases over the slick. The colloidal fraction for the slick soil is about three times that of the normal soil. This varies inversely with the Ca:Na ratio in the base-exchange complex.

TABLE 6

*Dispersion of clayic acid and various clayates**

SOIL	ACIDOID OR SALOID	CLAY CONTENT	D. F.	D. C.
		per cent	*per cent*	*per cent*
Hoos fallow 18–27″	Clayic acid	33.9	1.0	2.9
	Na clayate	35.56	34.9	98.1
Rothamsted	K clayate	30.8	3.1	10.1
	Ca clayate	31.1	3.6	11.6
	Ba clayate	32.1	3.7	11.5
	Al clayate	32.3	1.7	5.3
Dhrwar soil	Clayic acid	60.5	1.4	2.3
	Mg clayate	60.5	3.2	5.3
	Ca clayate	60.5	2.2	3.6
	Ba clayate	60.5	2.6	4.3
	Al clayate	60.5	0.5	0.8
Ahola soil	Clayic acid	60.6	2.5	4.1
	Ca clayate	60.6	3.0	4.9
	Ba clayate	60.6	3.2	5.3

* Table 1 from Puri (23).

Puri (23) in his studies of factors influencing the dispersion of soil colloids in water derives two factors—the dispersion factor and the dispersion coefficient. He states, "the degree of dispersion was measured by determining the percentage of conventional clay (0.002 mm. diameter) by the pipette method after leaving the soil in contact with water for 24 hours (referred to as dispersion factor or D. F.) and expressing it as percentage on the total clay content of the soil obtainable on complete dispersion. This is referred to as dispersion coefficient (D. C.) or D. C. $= \frac{\text{D. F.} \times 100}{\text{Clay Content}}$."

The dispersion factor was determined in the samples used in this study (table 5). The results obtained from this study were closely correlated with the ratio of exchangeable Ca:Na in the soils. However, when the D. C. was

determined, using the colloidal clay for the soil from table 4, somewhat erroneous results were obtained. To illustrate the point, table 1 from Puri's paper (23) is given as table 6.

In this table he finds that the Na Clay has a D. C. of almost 100 per cent, and that Ca and other clays have a very low D. C. Singh and Nijhawan (26) find somewhat the same relation. They make the statement, "it seems feasible to seek for a relationship between the dispersion coefficient of soils under examination and the Ca:Na ratio in the exchange complex." In our work a different relation was found.

The slick soil has a higher D. F. than a normal soil, but the decrease of clay for the normal soil over the slick soil in proportion to the D. F. for those two soils is much larger for the normal soil. For that reason the D. C. proves to be larger for the normal soil, whereas according to Puri's work it should be smaller.

It can be seen, then, that the D. C. might give an erroneous idea of the physical state of the soil. The D. F., on the other hand, expresses the condition of the soil very well, and gives a simple and rapid way of classifying a soil.

SUMMARY

A set of soils was made up to study the relation among plant growth, water consumed by the plants, ratio of exchangeable Ca:Na in the soils, and the mechanical composition of the soil.

Chemical analyses and pH determinations made on slick and normal soils show very little difference between those two soils; they are also low in water-soluble salts.

There is a marked difference in the Ca:Na ratio in the exchange complex of the soils studied. The ratio for slick soil is low, 1.71; for the normal soil, very high, 48.40.

The mechanical analysis of the soils made shows that the colloidal fraction of a slick soil is about three times as great as that of a normal soil.

The amount of water necessary to produce 1 gm. of dry matter for the slick soil is about three times that for the normal soil.

Low crop producing power of the slick soil is attributed to the poor physical condition of this soil, which in turn is influenced by a relatively high exchangeable Na content.

The deflocculation factor of Puri expresses the physical condition of a soil; the dispersion coefficient runs contrary to what was expected.

REFERENCES

(1) ANDERSON, M. S. 1929 The influence of substituted cations on the properties of soil colloids. *Jour. Agr. Res.* 38: 565–584.

(2) BAVER, L. D. 1929 The effect of the amount and nature of exchangeable cations on the structure of a colloidal clay. Mo. Agr. Exp. Sta. Res. Bul. 129.

(3) BENNETT, H. H. 1925 Some comparisons of the properties of humid tropical and humid-temperate American soils. *Soil. Sci.* 21: 349–375.

(4) BOTKIN, C. W. 1927 The effect of the constituents of alkali, fertilizers, and soil amendments on the permeability of certain fine-textured soils under irrigation. N. M. Agr. Exp. Sta. Tech. Bul. 160.

(5) BREAZEALE, J. F., AND MCGEORGE, W. T. 1926 Sodium hydroxide rather than sodium carbonate the source of alkalinity in black alkali soils. Ariz. Agr. Exp. Sta. Tech. Bul. 13.

(6) BURGESS, P. S., AND BREAZEALE, J. F. 1926 Methods for determining the replaceable bases of soils, either in the presence or absence of alkali salts. Ariz. Agr. Exp. Sta. Tech. Bul. 9.

(7) CUMMINS, ARTHUR B., AND KELLEY, WALTER P. 1923 The formation of sodium carbonate in soils. Calif. Agr. Exp. Sta. Tech. Paper 3.

(8) GEDROIZ, K. K. 1912 Colloidal substances in the soil solution. Formation of sodium carbonate in the soil. Alkaline soils and saline soils. *Zhur. Opytn. Agron.* [Russian] 13: 363–412. (English translation by Waksman.)

(9) GEDROIZ, K. K. 1913 Rapidity of reaction exchange in the soil. The colloidal condition of the soil saturated with various bases, and the indicator method of determining the colloidal content of the soil. *Zhur. Opytn. Agron.* [Russian] 15: 181–205. (English translation by Waksman.)

(10) GEDROIZ, K. K. 1923 The hydrochloric acid method for determining in the soil the cations present in an absorbed condition. *Soil Sci.* 16: 473.

(11) GEDROIZ, K. K. 1931 Exchangeable cations of the soil and the plant: I. Relation of plant to certain cations fully saturating the soil exchange capacity. *Soil Sci.* 32: 51–63.

(12) GEDROIZ, K. K. 1932 The absorption complex, plants, fertilization and melioration. *Chimizatziya Soc. Zeml.* [Russian] 1: 40–49.

(13) HISSINK, D. J. 1922 Beitrag zur Kenntnis der Adsorptionsvorgaenge im Boden. *Internatl. Mitt. Bodenk.* 12: 123–136.

(14) HISSINK, D. J. 1923 Method for estimating absorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269–276.

(15) HISSINK, D. J. 1925 Base exchange in soils. *Trans. Faraday Soc.* 20: 551–566.

(16) KELLEY, W. P., AND BROWN, S. M. 1924 Replaceable bases in soils. Calif. Agr. Exp. Sta. Tech. Paper 15.

(17) KELLEY, W. P., AND BROWN, S. M. 1925 Base exchange in relation to alkali soils. *Soil Sci.* 20: 477–495.

(18) MATTSON, S. 1926 The relation between the electrokinetic behavior of the base exchange capacity of soil colloids. *Jour. Amer. Soc. Agron.* 18: 458–470.

(19) MATTSON, S. 1926 The electrodialysis of the colloidal material and the exchangeable bases. *Jour. Agr. Res.* 33: 553–567.

(20) MATTSON, S. 1928 The influence of the exchangeable bases on the colloidal behavior of soil materials. *First Internatl. Cong. Soil Sci. Proc.* 2: 185–198.

(21) OLMSTEAD, L. B., ALEXANDER, L. T., AND MIDDLETON, H. E. 1930 A pipette method of mechanical analysis of soils based on improved dispersion procedure. U. S. Dept. Agr. Tech. Bul. 170.

(22) PETERSON, P. P. 1918 The slick spots of middle western Idaho with suggestions for their elimination. Idaho Agr. Exp. Sta. Bul. 114.

(23) PURI, A. M. 1930 Factors influencing the dispersion of soil colloids in water. *Mem. Dept. Agr. India Chem. Ser.* 11 (2): 39–51.

(24) PURI, A. M. 1931 A simple method of estimating total exchangeable bases in soils. *Soil Sci.* 31: 275–279.

(25) SCHOLLENBERGER, C. J., AND DREIBELBIS, F. R. 1930 Analytical methods in base exchange investigations on soils. *Soil Sci.* 30: 161.

(26) SINGH, D., AND NIJHAWAN, S. D. 1932 A study of the physico-chemical changes accompanying the process of reclamation in alkali soils. *Indian Jour. Agr. Sci.* 2: 1–18.

INFLUENCE OF EIGHT YEARS' SUCCESSIVE FERTILIZING WITH CONCENTRATED POTASH SALTS AND WITH KAINIT ON THE REPLACEABLE BASES OF THE "TERRA ROXA" SOIL

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Received for publication July 7, 1933

The study of the replaceable cations of the soil has been the subject of important papers by Gedroiz (3), Hissink (8), Ramann (21), Kappen (13), Kelley (15), Wiegner (23), de Sigmond (22), Page (18), Gehring (6), and many others. These studies point out very clearly the influence of the replaceable base content of the soil on its physicochemical and biological properties and therefore also on its fertility.

In this paper are published the results obtained in several experiments designed to determine the influence of potassium chloride, potassium sulfate, kainit, sodium chloride mixed with magnesium sulfate, and magnesium sulfate alone on the replaceable bases of the "Terra Roxa"[1] soil of our experimental plots (Santa Eliza Farm) which have been fertilized in the same way for 8 successive years.

PRELIMINARY INFORMATION

The experimental plots for the study of the effect of potash fertilizers at Santa Eliza Farm were established November, 1925, on an exhausted soil very poor in nutritional elements but which, because of its good physical properties and its favorable topography, suited our purpose very well.

The chemical composition of this "Terra Roxa" is as follows:

ORGANIC MATTER	H_2O AT 110°C.	SiO_2	TiO_2	Fe_2O_3	Al_2O_3	MnO	CaO	MgO	K_2O	Na_2O	P_2O_5	SO_3	Cl
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*
10.94	3.25	37.52	5.37	21.95	19.56	0.11	0.29	0.05	0.11	0.94	0.11	Trace	0.02

Its physical constitution is as follows:

SAND 1–0.2 MM.	FINE SAND 0.2–0.04 MM.	SILT 0.04–0.01 MM.	FINE SILT 0.01–0.002 MM.	CLAY + SUBSTANCES BELOW 0.002 MM.	HUMUS	MOISTURE CONTENT
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
16.0	13.05	0.40	15.64	30.07	2.12	15.71

[1] Red soil resulting from the decomposition of augite-porphyretic rocks (1).

The experimental ground was divided into a series of plots 5 by 10 m., each plot being repeated six times in the field.

The fertilizers applied to the plots of the different series are shown in table 1.

The nitrogen and phosphoric fertilizers were always applied in the form of nitrate of soda and superphosphate (18 per cent) respectively. Since 1925

TABLE 1

Fertilizer treatment of plots

Each plot measured 50 sq. m.

SERIES NUMBER	K_2O	P_2O_5	N	CaO	MgO	Na_2O	REMARKS
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
1	...	...	...	.....	...	...	
2	...	450	400	7,980	...	...	The calcareous fertilizers were applied October, 1927, and in 1929
3	350	450	400	7,980	...	...	The K_2O was applied in form of KCl
4	350	450	400	7,980	...	...	The K_2O was applied in form of K_2SO_4
5	350	450	400	7,980	143	518	The K_2O was applied in form of kainit
6	350	450	400	7,980	139	...	The K_2O was applied in form of potassium and magnesium sulfate
7	...	450	400	7,980	143	518	The MgO was applied in form of $MgSO_4 + 7H_2O$ and the Na_2O in form of NaCl
8	...	450	400	7,980	143	...	The MgO was applied in form of $MgSO_4 + 7H_2O$

TABLE 2

Averages of 40 years' meteorological records for São Paulo

Average temperature	19.7°C.
Vapor pressure	17.0 mm. Hg
Relative humidity	75.2
Relative deficit of saturation (100–75.2)	24.8 per cent
0.248 x 17.04 mm. Hg	4.22 mm. Hg
Rainfall	1,409.1 mm.
Quotient $\frac{N}{S}$	333.8
Average of maximum temperatures	26.2°C.
Average of minimum temperatures	14.5°C.
Absolute maximum temperature (14–10–901)	36.7°C.
Absolute minimum temperature (14–7–92 and 19–8–902)	0.2°C.

the following crops have been cultivated on these experimental grounds: corn, barley, corn, barley, cotton, corn, wheat, cotton, wheat, and rice. Before sowing each of these crop plants, we always applied the quantities of fertilizers indicated in table 1.

CLIMATE

The climate of Campinas, which is more or less characteristic for the plateau of the State of São Paulo, can be distinctly divided into two different periods: one is dry and temperate (April to September) and the other is hot and moist (October to March).

The figures given in table 2, which represent average data of 40 years' observations at the Metereological Observatory of this Agronomical Institute, indicate very clearly the characteristics of our climate.

The quotient $\frac{N}{S}$ proposed by A. Meyer (17) gives an approximate idea of the humidity of a given region. A high coefficient indicates a humid climate, and a low one a dry climate. In order to obtain this quotient it is necessary to look up on a table of physical constants the vapor pressure which corresponds to the average annual temperature of the region. The figure thus obtained, expressed in millimeters of Hg, multiplied by the relative saturation deficit, gives us the absolute saturation deficit, which, divided by the quantity of rainfall during the year, expressed in millimeters, gives us the coefficient $\frac{N}{S}$.

SOIL SAMPLING

The soil samples used in this study were taken at several places from the plots which make up a replication series, at a depth of 30 cm.; they were very well mixed together, passed through a 1-mm. mesh sieve, and then stored in hermetically closed glass bottles.

EXPERIMENTAL METHODS

The pH values were determined in the aqueous suspension of the soil by the electrometrical method with a quinhydrone electrode; the exchange acidity was determined by the method of Daikuhara (2) and the hydrolitic by the method of Kappen.

The "buffer" capacity, which Kappen (12, p. 364) defines as the resistance of the soil to a change of reaction when one adds to it an acid or a base, was determined by the method of Jensen (11). In each of eleven 100-cc. Pyrex cylinders was placed 10 gm. of air-dried soil which had been passed through a 1-mm. mesh sieve. To cylinder No. 0 was added 10 cc. of boiled water, and to the others, 1 to 10 cc. of 0.1 *N* HCl or 0.1 *N* $Ca(OH)_2$ the volume being completed with distilled and boiled water. All the cylinders were plugged with rubber stoppers and left undisturbed for 24 hours. Afterwards the pH values were determined for each of them.

In order to present graphically the neutralization curve of each of the soils, we drew graphs locating on their abscissas the quantities of acid or of base added to each of the tubes expressed in cubic centimeters, and on the ordinates the pH values found in each case. For comparative purposes we drew for

each soil a second curve following the aforementioned method, taking instead of soil very find pure sand, very well washed with acid.

The area located between these two curves, measured with a planimeter and expressed in square centimeters, represents the "buffer" surface of the soil.

"Buffer number" represents the distance measured on the abscissa between the bases of two perpendicular lines, the first of which passes through the point

TABLE 3

Hydrogen-ion concentration of soil samples treated with different quantities of 0.1 N *HCl*

NUMBER OF THE PLOTS	HYDROGEN-ION CONCENTRATION OF SOIL SAMPLES TREATED WITH										
	0 cc. 0.1*N* HCl	1 cc. 0.1*N* HCl	2 cc. 0.1*N* HCl	3 cc. 0.1*N* HCl	4 cc. 0.1*N* HCl	5 cc. 0.1*N* HCl	6 cc. 0.1*N* HCl	7 cc. 0.1*N* HCl	8 cc. 0.1*N* HCl	9 cc. 0.1*N* HCl	10 cc. 0.1*N* HCl
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
7	6.72	6.52	5.72	5.36	4.81	4.45	4.11	3.91	3.54	3.36	3.09
8	6.57	6.23	5.46	5.03	4.61	4.32	3.93	3.60	3.42	3.19	3.04
10	6.52	6.03	5.22	4.54	3.85	3.47	3.16	3.02	2.98	2.82	2.61
11	6.56	6.33	5.37	4.86	4.53	4.10		3.49	3.21	3.12	2.89
12	6.60	6.08	5.20	4.73	4.22	3.80	3.47	3.23	3.11	3.02	2.79
13	6.45	6.39	6.01	5.44	5.12	4.76	4.46	4.36	3.95	3.75	3.48
14	5.82	4.96	4.08	3.65	3.23	3.00	2.83	2.80	2.64	2.50	2.41

TABLE 4

Hydrogen-ion concentration of soil samples treated with different quantities of 0.1 N *$Ca(OH)_2$*

NUMBER OF THE PLOTS	HYDROGEN-ION CONCENTRATION OF SOIL SAMPLES TREATED WITH										
	0 cc. 0.1*N* Ca $(OH)_2$	1 cc. 0.1*N* Ca $(OH)_2$	2 cc. 0.1*N* Ca $(OH)_2$	3 cc. 0.1*N* Ca $(OH)_2$	4 cc. 0.1*N* Ca $(OH)_2$	5 cc. 0.1*N* Ca $(OH)_2$	6 cc. 0.1*N* Ca $(OH)_2$	7 cc. 0.1*N* Ca $(OH)_2$	8 cc. 0.1*N* Ca $(OH)_2$	9 cc. 0.1*N* Ca $(OH)_2$	10 cc. 0.1*N* Ca $(OH)_2$
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
7	6.72	7.14	7.31	7.48	7.62	7.87	7.97	8.10	8.22	8.82	9.07
8	6.57	7.81	7.20	7.40	7.60	7.80	8.16	8.22	8.45	8.50	
10	6.52	6.88	7.10	7.32	7.52	7.75	7.98	8.21	8.23	8.69	8.70
11	6.56	7.03	7.15	7.40	7.55	8.20	8.26	8.35	8.45	8.63	8.87
12	6.60	6.95	7.19	7.24	7.81	8.12	8.43	8.65	8.99	9.07	9.19
13	6.45	6.99	7.10	7.26	7.60	8.23	8.42	8.48	8.60	8.74	8.77
14	5.82	6.52	6.88	7.03	7.30	7.66	7.93	8.00	8.10	8.27	8.47

where the parallel to the abscissa, traced from the extremity of the soil curve, meets the basic curve, the other of which starts at the beginning of the soil curve.

Kappen (12, p. 317) thinks that the method of Jensen gives good results for determining the "buffer capacity" of the soil when one wants to find only the "buffer surface"; he believes, however, that the "buffer number" thus obtained, does not always show up the real buffer capacity and the other acidity values of the soil.

The determinations of the "buffer capacity" of the soil from the different plots, made as mentioned, revealed the pH values shown in tables 3 and 4, which were used to draw the neutralization curves and to calculate from them the "buffer surface" of each of them.

The total bases were determined by the methods of Gedroiz (4) and Hissink (9), and the saturation capacity of the soil by the methods of Hissink (10, p. 137) and Gehring, Pegau, and Wehrmann (7). We determined the quantity of Ca adsorbed by the soil, as Hissink proposes, as follows: 25 gm. of fine soil was placed in a 200-cc. beaker to which was added 100 cc. of a normal solution of NaCl. It was left to digest for 24 hours in an oven kept at 60°C. The following day the liquid of the beaker was thrown on a filter to be drained into a 1-liter graduated flask, then all the soil was quantitatively transferred to the filter. The residue was then leached with successive portions of a normal solution of NaCl (cold), until 1,000 cc. of filtrate was obtained to fill the flask. The filter was then removed to another liter flask, and the leaching with the normal solution of NaCl was continued until this second flask was filled.

TABLE 5

Buffer surface and buffer number of soil samples from the experimental plots

	NUMBER OF THE PLOTS						
	7	8	9	10	11	12	13
Buffer surface	11.1	10.0	4.6	7.2	8.9	8.2	11.7
Buffer number	8.8	8.7	6.4	7.2	8.6	8.4	9.0

In the first liter we find all the calcium adsorbed and a small portion in the form of carbonate; the difference between the quantities of calcium found in the first and second liter flask gives us the quantity adsorbed by the soil. In the present experiment the quantity of calcium found in the second liter flask was insignificant.

The determination of the maximum quantity of bases which a given soil is capable of adsorbing was made by the method of Gehring, Pegau, Wehrmann (5) as follows: 25 gm. of fine soil was added to 100 cc. of a saturated solution of calcium hydroxide and heated to 60°C. The thermometer used to measure the temperature was washed with 5 cc. of distilled water, which was added to the liquid in contact with the soil, the volume of which was thus increased to 105 cc. After the flask had been left undisturbed for 24 hours, to the liquid was added a few drops of phenolphthalein, which should tinge the liquid red. This did not happen, however, because the soil had adsorbed all the calcium from the 100 cc. of $Ca(OH)_2$ of the first addition. Twenty cubic centimeters more of the saturated solution of calcium was added, and the flask was again left undisturbed. Afterwards some CO_2 gas was passed through the liquid until its color changed from red to colorless. Then the soil suspension was heated for some time, enough NaCl being added to keep a normal solution. The

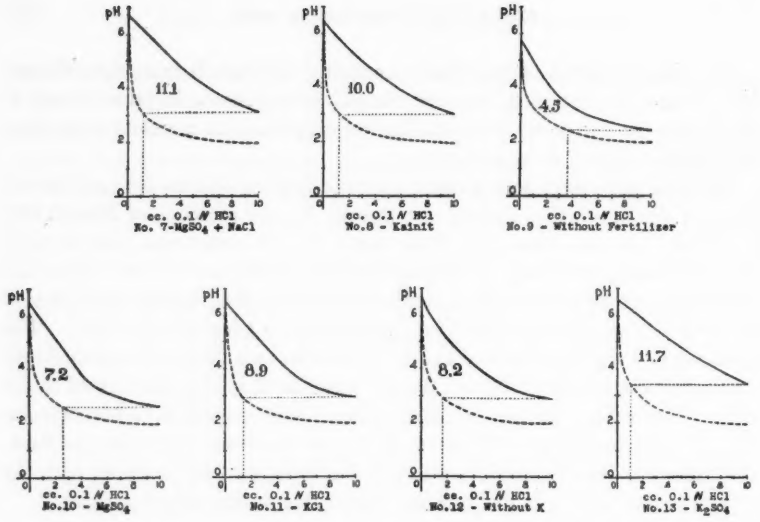

FIG. 1. GRAPHS OF BUFFER SURFACE AND BUFFER NUMBERS OF SOIL SAMPLES TREATED WITH DIFFERENT QUANTITIES OF TENTH-NORMAL HYDROCHLORIC ACID

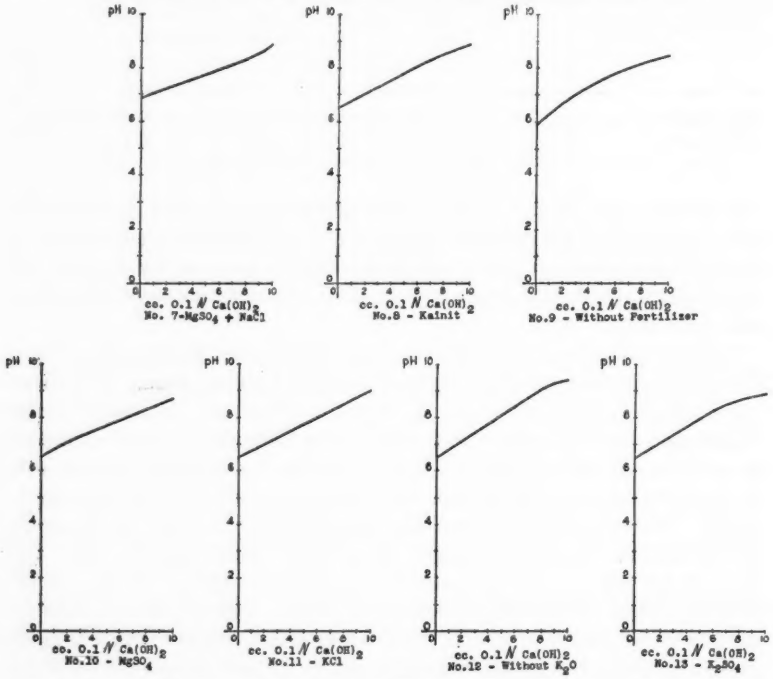

FIG. 2. THE PH VALUES OF SOIL SAMPLES TREATED WITH DIFFERENT QUANTITIES OF TENTH-NORMAL CALCIUM HYDROXIDE

TABLE 6

Comparison of exchangeable bases in soil samples from the differents plots

	NUMBER OF THE PLOTS	Al_2O_3		CaO		MgO		MnO		Na_2O		K_2O	
		mgm.	*m.e.*	*mgm.*	*m.e.*	*mgm.*	*m.e.*	*mgm.*	*m.e.*	*mgm.*	*m.e.*	*mgm.*	*m.e.*
$NaCl + MgSO_4$	7	0.0954	5.618	0.3123	11.140	0.0254	1.253	0.0237	0.622	0.0364	1.174	0.0107	0.227
Kainit	8	0.1006	5.923	0.3038	10.835	0.0195	0.963	0.0315	0.828	0.0381	1.229	0.0176	0.373
$MgSO_4$	10	0.0968	5.695	0.2833	10.106	0.0211	1.052	0.0249	0.654	0.0339	1.094	0.0097	0.205
KCl	11	0.0998	5.873	0.2904	10.358	0.0133	0.658	0.0310	0.801	0.0371	1.196	0.0195	0.412
Without K_2O	12	0.099	5.927	0.2938	10.479	0.0137	0.681	0.0308	0.808	0.0335	1.081	0.0095	0.201
K_2SO_4	13	0.0965	5.680	0.3249	11.592	0.0158	0.785	0.0312	0.818	0.0339	1.094	0.0196	0.406

TABLE 7

Value for T, S, T-S, $100\ \frac{(T\text{-}S)}{T}$, *and* $V = \frac{100.S}{T}$ *as determined by the method of Hissink modified by Gehring, Pegau, Wehermann*

	NUMBER OF THE PLOTS	HYDROLYIC ACIDITY y_1	ACIDITY DAIKUHARA y_1	pH	CLAY + FINE SILT	MAXIMUM SATURATION CAPACITY T		REPLACED Ca S		SATURATION DEFICIT T-S		SATURATION DEFICIT $\frac{(T\text{-}S)}{T}$	$\frac{S}{\text{CLAY + FINE SILT}}$	DEGREE OF SATURATION $\frac{V\text{-}100\times S}{T}$
						CaO	m.e.	CaO	m.e.	CaO	m.e.			
												per cent		*per cent*
$NaCl + MgSO_4$	7	.....	....	6.72	47.11	0.4485	16.02	0.2702	9.65	0.1783	6.37	39.81	0.20	60.2
Kainit	8	11.83	0.14	6.57	48.70	0.3869	13.82	0.2668	9.53	0.1201	4.29	31.03	0.19	69.0
$MgSO_4$	10			6.52	49.70	0.5384	19.23	0.2742	9.79	0.2642	9.43	48.99	0.19	50.9
KCl	11	13.31	0.13	6.56	46.61	0.3802	13.58	0.2789	9.96	0.1013	3.62	26.65	0.21	73.3
Without K_2O	12	11.55	0.20	6.60	46.21	0.5630	20.11	0.2707	9.67	0.2923	10.44	50.91	0.20	48.0
K_2SO_4	13	12.14	0.15	6.45	49.18	0.5807	20.74	0.2833	10.12	0.2974	10.62	51.20	0.20	48.8

liquid was then decanted and the soil was placed quantitatively on a filter above a liter flask and leached with a normal solution of NaCl (cold) until 1,000 cc. of the filtrate was obtained. Afterwards the filter with the soil was transferred to a second liter flask, and the leaching continued until the flask was filled. The quantities of calcium in the first and in the second liter flasks were determined. The difference between these quantities is the maximum quantity of calcium which the soil can adsorb (tables 6 and 7).

In order to calculate the degree of saturation of the soil, Hissink (10, p. 143) uses the following formula $V = \frac{100\,S}{T}$, in which V represents the saturation capacity of the soil in percentage, T the maximum quantity of a base which a soil can adsorb, and S the quantity of adsorbed calcium which the soil contains.

DISCUSSION

The continued use of potassic fertilizers, intensifying the concentration of K cations in the soil solution, modifies the relation among the adsorbed bases. This occurs because the potassium replaces these bases from the humic and zeolitic compounds of the soil, making them much more readily exchangeable, as seen in table 8.

As specified in the table, the dominant base is calcium, with the maximum percentage of 84.2 per cent for the series, without potash. Sodium takes second place with the maximum percentage of 9.4 per cent in the potassium chloride series.

It is noteworthy that the application of sodium chloride did not increase the percentage of adsorbed sodium, as might have been expected, for the soil samples which did not receive sodium chloride contained as much adsorbed sodium as the others which did get an addition of this salt. The anion of the salt proved to have a great influence, as the potassium sulfate series contains 2.3 per cent less sodium than the chloride series.

The series which received magnesium, on the contrary, showed greater content of adsorbed magnesium than the series which did not receive it. Nearly all the series without magnesium show almost the same proportion of this salt, that is, 5.2 per cent. It is also very interesting to follow the way in which the potassium acts in the soil. All series which received potassic fertilizers contained greater proportions of this cation in the adsorbed state than did those without potassium. But the series in which the salt was applied in the form of chloride contained a greater proportion of potassium than did the sulfate series.

Adsorbed bases and state of saturation

A comparison of the chemical analyses of the soil samples from the plots of the chloride, potassium, sulfate, and kainit series shows that the percentage of adsorbed calcium varies as follows: 0.293 per cent in the series without potassium; 0.303 per cent in the series with kainit; 0.290 per cent in the series with chloride; 0.325 per cent in the series with potassium sulfate.

The influence of the anion of the salt is very clear in this case, that is, the series which received chloride contained less adsorbed calcium than the ones which received sulfate. On the contrary, Page and Williams (19) found no considerable differences in the percentage of replaceable calcium among the various plots of Broadbalk which have been annually fertilized now for 82 years, probably because the soil is well supplied with calcium.

But small differences were noted between the influences of kainit and chloride; the amount of adsorbed calcium found in the soil of the plots which received such potassic fertilizers is approximately the same as that found in soil samples of plots without potassic fertilizer applications.

Similar observations were made concerning magnesium.

Noteworthy, also, is the following observation: Studying the changes of the capacity of the soil to adsorb bases, this experiment showed that this capacity of the soil in different series increased very considerably in 2 years. In 1929 the plots of the series of chloride, kainit, and without potassium showed re-

TABLE 8

Relative proportions of different bases in plots receiving several kinds of fertilizers

NUMBER OF THE PLOTS	TOTAL BASES	Ca	Mg	Na	K
	mgm.	*per cent*	*per cent*	*per cent*	*per cent*
7	13.79	80.7	9.08	8.5	1.6
8	13.40	80.8	7.20	9.1	2.7
10	12.45	81.2	8.40	8.7	1.6
11	12.60	82.07	5.20	9.4	3.23
12	12.44	84.2	5.30	8.6	1.60
13	13.88	83.5	5.60	7.1	2.90

spectively the following percentages of total bases: 6.49, 5.64, 6.24, and 6.66 m.e., as determined by the Bobko and Askinasi method. After the experiment these plots contained respectively 12.6, 13.88, 13.4, and 12.44 m.e., as determined by the Gedroiz method. Thus the increases are: for the potassium chloride series 6.11 m.e., for the sulfate series 8.24, for the kainit 7.16, and for the series without potassium 5.78 m.e.

The soils from the potassium chloride series contain less adsorbed magnesium than do those from the sulfate series. The soil samples from the chloride series are richer in sodium than are those of the sulfate series. Both series have, however, the same percentage of adsorbed potassium, as shown in table 8.

It is interesting to compare the percentages of adsorbed calcium, magnesium, sodium, and potassium existing in the soils of the sodium chloride plus magnesium sulfate series and of the kainit series with the soil sample from the series which received only magnesium sulfate. We noted approximately the same percentage of calcium in all the series. This did not occur with the replaceable magnesium and sodium, which were found in greater proportion in plots which

received an application of these salts. Neither the addition of magnesium nor of sodium had any influence on the percentage of adsorbed potassium, which remained approximately the same in the soil of all plots which did not receive potassic fertilizer.

Potassic fertilizer, as we have seen, showed a certain influence on the adsorbed bases, modifying also the capacity of the soil to adsorb bases and consequently also its state of saturation. It is interesting to note that the most saturated soils are those in the series receiving potassium chloride, 73.3 per cent, and the least saturated are those in the series without potassium, 48 per cent (table 7). The anion SO_4 apparently contributes toward the lowering of the saturation state of the soil.

Influence of potassium fertilizers on soil acidity

The potassium salts employed as fertilizers are physiologically acid, according to A. Mayer (16), because the cations are generally absorbed by the roots of the plants, the anions remaining in the soil. The continued use of these salts, therefore, on the same soil should render it acid after a certain period of time. This, however, does not happen, as several experiments have shown. Kappen (14) explains this fact by assuming that the plants adsorb a great deal of the anions from the employed fertilizers. He found in his experiments that plants absorbed about 50 per cent of Cl from the chlorides added to the soil. Prianischnikow (20) concluded that the plants differ markedly in this respect; he found, for instance, that oats and barley absorb cations and anions at approximately the same rate, whereas the maize plant absorbs nearly three times as many cations as anions.

Prianischnikow's conclusions are thus not in agreement with those of Kappen's. Furthermore, it must be kept in mind that the phenomenon of absorption in the soil is quite different from that in a nutrient solution. In the soil the bases are replaced in equivalent quantities, following the mass law, and the adsorption of one is always accompanied by the replacement of another or by hydrogen which is found in the absorption complex of unsaturated soils.

In our experiments the series of plots which received no potassium fertilizer but which was supplied with the same quantities of superphosphate, sodium nitrate, and calcium, has approximately the same pH index as the plots which received an application of potassium chloride and kainit. But this series which received no potassium is much less acid than the soil of the series which received potassium sulfate, as we see in table 7; the series without potassium has a pH index of 6.6, whereas the soil samples of the sulfate series have a pH index of 6.45, which shows them to be more acid. The hydrolitic acidity is a bit stronger in the series which received the potassium chloride and sulfate, but the Daikuhara acidity is weaker in both series.

SUMMARY

In this paper is discussed the influence of 8 years' successive fertilizing with concentrated potash salts and with kainit on the replaceable bases of the "Terra Roxa" soil.

A considerable increase in the soil's capacity to adsorb bases was revealed in the soils of all the experimental plots; this increase was most significant in the soils of the potassium sulfate series, followed by lower increases respectively in the series of kainit, potassium chloride, and without potassium.

It was also shown that the soil from the plots which received potassium chloride and kainit contained a higher proportion of adsorbed potassium than the soil from the sulfate series.

The soil in the potassium chloride series is more saturated with bases (73.3 per cent), whereas that in the potassium sulfate series contains only 48.8 per cent of bases.

We may conclude, therefore, that the anion of the salt plays a very important rôle in the effect of potash fertilizers in our "Terra Roxa" soil. The sulfates contribute more than the chlorides to decrease the state of saturation of the soil.

REFERENCES

(1) CAMARGO, THEODURETO DE, AND MELLO, P. C. 1927–28 Vergleichsstudium über den Saturationszustand und die austauschbaren Basen der Roten Erde im Urzustand und in 30 jähriger Kaffeekultur. *Landw. Vers Sta.* 107: 188–206.

(2) DAIKUHARA, G. 1914 *Bul. Imp. Cent. Agr. Exp. Sta. Japan* 2 (1): 19.

(3) GEDROIZ, K. K. 1918 On the method of determining the zeolitic bases in soils. *Russ. Jour. Exp. Landw.* 19: 226–248.

(4) GEDROIZ, K. K. 1929 Der absorbierende Bodenkomplexl. Theodor Steinkopff, Berlin.

(5) GEHRING, A. 1929 Über den Kalk und Kalisättigungsgrad des Bodens. *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* 13: 1.

(6) GEHRING, A. 1931 *Blanck's Handb. der Bodenlehre.* 8: 106.

(7) GEHRING, A., PEGAU, A., AND WEHRMANN, O. 1927 Über die Methoden zur Bestimmung des Kalkbedürfnisses des Bodens, etc. *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* 8 (A): 321–331.

(8) HISSINK, D. J. 1922 Beiträge zur Kenntnis der Absorptionsvorgänge im Boden. *Internatl. Mitt. Bodenk.* 12: 81–171.

(9) HISSINK, D. J. 1923 Method for estimating adsorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269–276.

(10) HISSINK, D. J. 1925 Der Sättigungszustand des Bodens. *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* 4 (A): 137–158.

(11) JENSEN, S. T. 1924 Über die Bestimmung der Pufferwirkung des Bodens. *Internatl. Mitt. Bodenk.* 14: 112–130.

(12) KAPPEN, H. 1931 *Blanck's Handb. der Bodenlehre.* 8: 317, 364.

(13) KAPPEN, H., AND LIESEGANG, H. 1921 Weitere Untersuchungen zur Austauschazidität der Mineralböden. *Landw. Vers. Sta.* 99: 191–230.

(14) KAPPEN, H., AND LUKACS, M. 1924 Zur physiologischen Reaktion der Düngungssalze. *Ztschr. Pflanzenernähr, Düngung, u. Bodenk.* 5 (A): 249.

(15) KELLEY, W. P., AND BROWN, S. 1924 Replaceable bases in soil. Calif. Agr. Exp. Sta. Tech. Paper 15.
(16) MAYER, A. 1901–2 Lehrbuch der Agrikulturchemie, pt. 2, ed. 3, p. 97. Heidelberg.
(17) MEYER, A. 1926 *Chem. der Erde.* 2 (2): 209–344.
(18) PAGE, H. J., AND WILLIAMS, W. 1925 Studies in base exchange in Rothamstead soils. *Faraday Soc. Trans.* 20: 573.
(19) PAGE, H., AND WILLIAMS, W. 1926 The effect of flooding with sea-water on the fertility of the soil. *Jour. Agr. Sci.* 16: 551–573.
(20) PRIANISCHNIKOW, D. N. 1931 Der Einfluss der Bodenreaktion auf das Pflanzenwachstum (Russian with German Summary). *Compt. Rend. Acad. Sci. U. S. S. R.*
(21) RAMANN, E., AND SPENGEL, A. 1919 Zur Kenntnis der Bodensorption. *Landw. Vers. Sta.* 92: 127–146.
(22) SIGMOND, A. A. J. DE 1926 *Verhandl. II Komm. Internatl. Bodenk. Gesell.* (Gröningen) 1926: 55, 71.
(23) WIEGNER, AND JENNY Über Basenaustausch an Permutiten. *Kolloid Ztschr.* 42: 268.

THE RELATION OF COLLOID DISPERSION IN SOILS TO CHEMICAL CHANGES INDUCED BY BIOLOGICAL TRANSFORMATIONS OF ORGANIC MATERIALS[1]

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Received for publication June 27, 1933

The effect of organic matter on the physical properties of soils has been abundantly demonstrated by practical experience and scientific experiment. Many of its influences, especially those of manure, were known to the ancients (16). Schübler has been credited by Keen (26) with being the first to show by laboratory experiments the effects of the presence of this material in the soil on its physical properties.

Warington (43), Russell (36), and Keen (26) discuss the beneficial effects of added organic matter on the physical properties of the soil. Keen attributes its favorable action to the improvement in soil tilth, rather than to a reduction in soil resistance, as measured by the draft of agricultural implements. Russell also attributes to humus a marked improvement in the tilth and general mechanical condition as well as an increase in moisture-holding capacity.

Many more citations, which would vary but slightly from the foregoing, could be given as to the beneficial effect of organic matter on the physical condition of the soil. There are, however, a few which can be interpreted to show that certain types of organic matter may have a much less beneficial, or even an injurious, effect. Sievers and Holtz (38) observed "the beneficial effects on the physical condition of the soil—generally resulting from the incorporation of organic matter in direct proportion to its nitrogen content." Legumes were found to be much more effective than non-legumes.

Chapman (13) reported that after seedbed preparation, only 10.8 per cent of the surface soil of the timothy (rotation: corn, barley, timothy, wheat) plot passed through a 1/16-inch mesh screen, whereas 55.7 per cent from the clover (rotation: corn, barley, clover, wheat) plot passed through it. The soil crumbs from the timothy plot were sharp-edged whereas, those from the other were rounded, irregular, and far from granular. To account for his results, he suggested three possible causes; namely, granulation on wetting and drying set up by the different stresses of organic and mineral soil material; weaknesses developed by the decay of the organic matter, producing lines of cleavage; and qualitative and quantitative differences in the concentration of ions in the soil brought about by the difference in plants grown. The last he considers the least likely.

Stephenson (39) in studying the effect of different organic materials on soil reaction found that albumin and casein added to soils and incubated made them hard, lumpy, and difficult

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[1] Contribution from the Division of Agronomy, University of California, Davis, California. Condensation of a dissertation, submitted in partial satisfaction of the requirements for the degree of doctor of philosophy in soil science in the Graduate Division of the University of California. The writer appreciates the helpful suggestions and considerate criticisms given during the progress of the investigations by the members of the committee in charge of his dissertation: Professors John S. Burd, chairman, G. B. Bodman, and C. B. Lipman. Thanks are also due Professors D. R. Hoagland and W. P. Kelley for suggestions regarding the manuscript. A number of the analyses reported herein were made by Mrs. D. P. Danno of the Division of Agronomy.

to handle even in the presence of lime. Suspensions from them passed through two thicknesses of filter paper. Additions of blood caused similar results in the early part of the experiment, but later the filtrates were clear. The addition of carbohydrate materials, especially starch, caused the soils to become sticky and "of a consistency similar to putty." The alfalfa-treated soils remained "granular and in good tilth."

Thus, it appears that not all types of organic matter have the same effect on the physical properties of a soil. Inasmuch as the chemical properties of the materials which cause such divergent physical conditions in the soil are so markedly different, it would seem that these substances can show their influence in at least two ways.

On the one hand, organic material operates directly or statically by its mere presence in the soil. The properties which it imparts in this regard are largely additive, i.e., they may be considered as a summation of the physical and colloidal properties of the organic substances and of the mineral soil particles, with due regard, of course, for size and aggregation of units. Its influence in this respect is felt as long as the organic material itself remains. Baver (4) removed a considerable part of the organic matter from soils by oxidation with peroxide according to Robinson's (35) method, thereby obtaining lower absorptive capacities for cations and for hygroscopic moisture as well as lower moisture percentages for the upper and lower plastic limits.

On the other hand, organic matter may influence the soil indirectly or dynamically by the changes which it induces. The properties which it imparts to the soil in this way have very little relation or none whatever to its own physical properties. Its effects are not additive to those of the mineral soil colloids but result from the microbiological activity caused by its presence in the soil. The compounds making up the organic matter, although they are themselves largely non-electrolytes, influence the physical properties by bringing about changes in the electrolyte concentration of the soil solution.

Organic materials resistant to decay largely exert their influence statiscally; those which quickly decay show their effects dynamically.

Much attention has been given to the rôle that electrolytes play in coagulating, flocculating, and dispersing soil colloids in clay suspensions. Recently Keen (26) has summarized them. Nitrification of organic materials and fallowing have increased electrolyte concentrations in soils (3, 10, 11, 12, 27, 41). Higher plants and microörganisms furnished with carbonaceous materials have reduced the electrolyte concentrations (10, 11, 12, 15, 23, 41). Sufficient change in the concentration of the soil solution, biologically produced, may alter the stability of the soil suspension. This has been sufficient, with higher plants at least, to produce changes in the dispersion of the soil colloids (2, 3, 17, 22, 31). With respect to the dynamic rôle of organic materials in soils, however, there is need for a study of the quantitative interrelationships of the rate of application of material, its chemical composition, duration of incubation, concentration of various ions, and the accompanying physical properties as applied to given soils.

PLAN OF THE EXPERIMENTS

The soils, a Fresno fine sandy loam from a vineyard in Fresno County and a Yolo loam from a fallowed field in the experimental area at Davis, were carefully prepared for laboratory work by drying, crushing of the larger clods, sifting, and thoroughly mixing. Of the varied amounts of urea added, the greatest was sufficient when fully nitrified to precipitate practically all of the colloids in suspension as measured by the procedure described in the following.

Samples of each of these soils were treated with 50 m.e. of HNO_3 per kgm. of dry soil in the case of the Fresno soil, and 25 m.e. in the case of the Yolo soil. Samples thus treated are referred to as "nitrated" Fresno fine sandy loam and "nitrated" Yolo loam respectively. After drying, each soil was again sifted

and thoroughly mixed. Sucrose was added to these samples in increasing amounts, the largest addition being sufficient to disperse the soil colloids materially.

The treatments for the various samples of soil were as follows:

SOILS	TREATMENTS					
	Ucea—m.a. of nitrogen					
Fresno fine sandy loam.....	0	10	20	35	60	
Yolo loam.....	0	4	8	15	30	
	Sucrose—m.a. of carbon					
Fresno fine sandy loam "nitrated".	0	75	150	300	750	
Yolo loam "nitrated".....	0	50	100	200	400	750

* Milligram-atoms per kilo of soil.

Samples were incubated in a room thermostatically controlled at about 28°C.

The analyses reported in the various tables were made by methods generally used in such determinations, all being volumetric, gravimetric, or electrometric. The determinations of NH_4 and NO_3 ions in the *N* KCl extract at pH 1 were made as described by Russell[2] (36, p. 567). Silica was determined as usual except that the final volatilization with HF was omitted.

Methods for suspended and soluble solids

In the preliminary trials, many criteria for the physical properties of the soils were examined. None of them was so easy of manipulation or permitted such good duplication of results as that of suspended solids. Although Puri and Keen (34) noticed a reduction in the amount of suspended material when the soil was increased much over 2 per cent of the water, it seemed desirable on the basis of preliminary tests, to take a concentration of 10 per cent.

Twenty-five grams of dry soil (or its equivalent in moist soil) was churned with 250 ml. (less the water in the soil) of distilled water, previously kept at a temperature of 30°C. This mixing was accomplished in an Arnold #16 sanitary drink mixer, in which the propeller had been directly attached to the motor shaft, and the lower bearing of which had been removed. Part of the suspension was poured quickly into a long glass tube (23 mm. in diameter) and placed in a 30°C. constant temperature chamber for 24 hours. The remainder of the suspension was then thrown on a filter to obtain the extract used for the determination of soluble solids and specific conductance. Total solids in each case were determined by withdrawing 20 ml. of suspension with a pipette, the tip of which was held 10 cm. below the original surface of the liquid, evaporating and drying. Suspended solids equal total solids minus soluble solids.

[2] In the nitrate determinations after the first distillation more water was added and distillation continued to secure all of the nitrates.

The churning period was 5 minutes in each case. Longer churning increased the dispersion, as shown by Puri and Keen (34), Bradfield (6), and Bouyoucos (5), as well as by our own preliminary results. This length of time was arbitrarily chosen because it gave quite reproducible results, and was not too time-consuming.

Hydrogen-ion concentration

These determinations were made with the quinhydrone electrode in a bath at 25°C., on 1:1 soil suspensions with H_2O and with *N* KCl. In general, the tests with the aqueous suspensions were made in quadruplicate and those with the *N* KCl in triplicate. The results are expressed in terms of pH. Ten second values were used in making readings.

UREA TREATMENTS

The effects of the decomposition of urea on Fresno fine sandy loam as determined for the various incubation periods are given in table 1. No conspicuous differences in the dispersion are disclosed except where the largest amount of urea was added. Although the electrolyte content was considerably augmented as a result of incubation, it did not cause any clear-cut flocculation of the suspended solids, presumably because of the dispersing effect of the increasing concentration of OH ions and of the greater amount of NH_4 ions in the exchange complex. The failure to accumulate NO_3 ions with the larger applications of urea was clearly assignable to the inhibiting effect of the larger amounts of ammonia upon the nitrification processes.

In the amounts of suspended solids as given in the various tables in this paper, minor variations appear which cannot be correlated with changes in the concentrations of the various ions so far studied. These variations must be attributed to a factor, or factors, as yet undisclosed.

In the control soil and in that receiving 10 m.a. of nitrogen as urea, ammonification and nitrification went on normally. Initially the ammonia content increased, sending the pH up to 8.19 in the latter treatment. Subsequently, with the change of ammonia to nitrate, the pH was lowered below that of the control soil. With the change of urea to ammonia, the specific conductance increased to some extent, but the increase was still more marked with the transformation of ammonia to nitrates. With the larger amounts of urea, it is not so easy to tell what changes had been taking place in all respects. As the pH values were high for some time during the incubation period some of the nitrogen may have been lost from the containers by volatization as ammonia.

More complete analyses of the Fresno fine sandy loam with various amounts of urea added were made after 4 weeks of incubation. The results of these are reported in table 2. No new points are brought out with regard to the suspended solids. The specific conductance of the 1:10 extract increased with increasing amount of urea; that of the 1:1 extract also increased with the exception of the treatment with the largest amount.

The concentration of bicarbonates rose with increasing amounts of urea. The K ions increased from 0.82 m.e. per kgm. in the control treatment to 1.67, with a falling off to 1.28 in the highest urea treatment. Sodium ions and Mg

TABLE 1

Effect of the biological destruction of urea on Fresno fine sandy loam as measured at various times during the incubation

UREA MGM.-ATOM NITROGEN PER KILO	TIME OF INCUBATION WEEKS	1:10 = SOIL:H_2O RATIO			QUIN pH AT 25°C. 1:1 SUSPENSION		*N* KCl EXTRACT AT pH 1 M.E. PER KILO	
		Mgm. in 20 ml.		Sp. cond. Mhos × 10^{-4}				
		Susp'd solids	Soluble solids		H_2O	*N* KCl	NH_4	NO_3
0	3/7	26.5	1.4	0.84	7.33	6.36	1.06	0.6*
	2	25.8	2.6	1.02	6.68	6.16	0.35	1.5
	7	21.0	1.2	1.23	6.24	5.72	0.22	3.96
10	3/7	24.1	2.0	1.46	8.19	7.47	10.10	0.8
	1	25.5	1.4	1.60	6.70	6.24	5.81	2.5
	2	29.1	3.2	1.78	5.98	5.70	4.62	6.1
	4	18.8	4.2	2.02	5.32	4.99	1.29	8.40
	7	19.3	3.2	2.12	5.06	4.91	0.51	10.40
	16	20.4	3.2	2.32	5.28	4.96	0.80	13.00
20	3/7	26.2	2.0	2.00	8.48	8.09	19.7	0.9
	1	27.5	1.5	2.16	7.75	7.41	12.92	2.4
	2	26.3	2.9	2.26	6.54	6.14	10.72	6.2
	4	21.7	2.4	2.12	6.40	6.00	8.10	5.5
	7	20.7	2.5	2.11	5.82	5.57	6.31	8.92
	16	26.1	2.5	2.06	6.25	5.73	7.20	6.70
35	3/7	25.4	2.7	2.89	8.63	8.35	30.23	0.6
	1	27.5	2.0	2.88	8.53	8.23	27.02	0.6
	2	31.2	3.4	3.00	7.64	7.34	25.55	6.2
	4	22.7	2.7	3.00	6.56	6.28	14.55	6.8
	7	22.7	2.6	2.76	6.33	6.07	10.20	12.12
	16	28.0	2.7	2.77	6.50	6.07	14.25	8.20
60	3/7	29.7	3.1	4.20	8.73	8.53	57.2	0.4
	2	33.3	4.7	3.84	8.67	8.38	52.5	0.3
	7	32.4	3.9	3.17	8.65	8.33	43.4	0.52

* Values in the last column for NO_3 given to one decimal place may be slightly low, as in their determination no second distillation was run. On those expressed to two places, after the contents of the flask were boiled down, additional water was added, the distillate caught and titrated separately, and the value added to the first.

ions were affected similarly to K ions, whereas Ca ions were a little more variable and were decidedly decreased by the largest urea treatment. The marked reduction of Ca and Mg ions in the highest urea treatment is consistent with the formation and precipitation of $CaCO_3$ and $MgCO_3$ in the soil at the pH

values which occurred in the soil at that time. The increase in phosphate may be ascribed in part at least to the decline in the concentration of the Ca and Mg ions. The PO_4 ions diminished slightly with increasing amounts of urea, except in the case of the largest urea treatment, where it was higher. Sulfate ions increased slightly.

The results of a similar study of the effects of urea decomposition on Yolo loam are given in table 3. Here nitrification, even in the presence of larger

TABLE 2

Effect of the biological destruction of increasing amounts of urea upon Fresno fine sandy loam after an incubation period of 4 weeks at 28°C.

UREA ADDED, MGM.-ATOMS OF NITROGEN PER KILO SOIL	0	10	20	35	60
1:10—suspension:					
Susp. solids. mgm. in 20 ml	21.2	21.0	21.2	21.4	26.0
Extract—soluble solids	2.8	3.8	3.5	3.8	4.6
Sp. cond. Mhos $\times 10^{-4}$	1.22	1.79	2.18	2.91	3.69
Quin pH 1:1 suspensions H_2O	6.19	5.77	6.19	6.43	8.43
N KCl	5.72	5.55	5.85	6.12	8.01
Extract: 1:1 = soil:H_2O					
Sp. Cond. Mhos $\times 10^{-4}$	5.58	11.10	15.12	20.83	20.47
Ions—m.e./kilo:					
NH_4	0.24	1.18	4.20	7.94	17.52
K	0.82	1.18	1.44	1.67	1.28
Na	0.69	0.75	0.80	0.95	0.80
Ca	2.32	4.55	4.19	4.44	1.47
Mg	1.12	2.28	2.24	2.32	0.97
Total cations	5.19	9.94	12.87	17.32	22.04
HCO_3	0.52	0.70	1.06	1.92	13.90
Cl	0.67	0.73	0.78	0.67	0.70*
NO_3	2.84	7.30	9.92	14.56	5.22
SO_4	0.68	0.67	0.63	0.62	0.95
PO_4	0.60	0.57	0.44	0.45	0.95
Total anions	5.31	9.97	12.83	18.22	21.72

* The solution was too dark colored for titration. This value was assumed.

applications of urea, was not sufficiently inhibited to prevent almost complete transformation of the ammonia to nitrates during a period of 11 weeks. Transformations took place during the first 4 weeks of incubation, sufficient in amount with the treatment of 8 m.a. of nitrogen as urea, to cause a material reduction of the colloids in suspension. As shown in the table, the higher applications of urea for this and longer periods of incubation reduced the suspended solids practically to zero.

During the course of the incubation, the specific conductance of the 1:10

extract increased with time. With the control soil this tendency may be attributed to the formation of nitrates from the decomposition of the soil's original organic matter. In the other cases this was a factor which, also, became less and less proportionally as the amount of urea was increased. In all treatments, the specific conductance increased over the control by the trans-

TABLE 3

Effect of the biological destruction of urea on Yolo loam as measured at various times during the incubation

UREA MGM.-ATOM NITROGEN PER KILO	TIME OF INCUBATION WEEKS	1:10 = SOIL:H_2O RATIO			QUIN pH AT 25°C. 1:1 SUSPENSION		*N* KCl EXTRACT AT pH 1 M.E. PER KILO	
		Mgm. in 20 ml.		Sp. cond. Mhos × 10^{-4}				
		Susp'd solids	Soluble solids		H_2O	*N* KCl	NH_4	NO_3
0	1	103.2	1.0	1.02	7.46	6.62	0.31	1.2*
	2	93.6	1.2	1.17	7.16	5.77	0.26	2.46
	7	105.2	1.6	1.26	7.18	5.96	0.12	2.60
	11	100.6	1.8	1.19	7.14	6.01	0.24	3.70
4	1	93.5	1.8	1.27	7.34	6.46	1.98	2.7
	2	106.5	1.8	1.51	6.80	5.73	0.56	3.78
	4	94.0	2.5	1.58	6.87	5.89	0.36	6.30
	7	91.8	2.0	1.56	6.87	5.89	0.12	6.12
	11	87.1	2.5	1.58	6.87	5.90	0.48	6.60
8	1	102.1	2.0	1.40	7.32	6.53	5.05	3.1
	2	104.6	2.0	1.65	6.67	5.77	2.28	7.16
	4	17.7	3.4	1.94	6.63	5.80	0.21	9.49
	7	20.0	2.8	1.95	6.60	5.73	0.26	9.26
	11	12.3	3.2	1.96	6.60	5.72	0.36	8.90
15	1	98.0	1.7	1.60	7.57	6.82	11.22	2.7
	2	104.8	2.2	1.90	6.89	5.95	6.90	7.94
	4	2.8	4.6	2.62	6.33	5.66	1.22	15.10
	7	2.5	3.6	2.69	6.24	5.42	0.18	15.50
	11	0.4	5.3	2.76	6.36	5.47	0.30	16.54
30	1	87.8	1.8	2.10	8.26	7.34	22.60	2.3
	2	112.1	2.2	2.28	7.47	6.63	18.88	7.48
	7	−0.2	5.6	3.82	5.77	5.13	2.98	24.80
	11	−0.7	7.6	4.46	5.65	5.06	0.66	28.18

* See footnote to table 1.

formations of the urea to ammonia, and still further increased by the change of ammonia to nitrates. The pH increased with the transformations of urea to ammonia, and then progressively decreased below the initial pH with the progressive change to nitrates.

The results of more complete analyses of the Yolo loam at the end of 11 weeks of incubation are given in table 4. Here, even in the highest urea treatment,

practically all of the NH_3 was transformed to nitrates. At 11 weeks, the nitrification of 15 m.a. of N (and higher rates of application) as urea caused practically complete flocculation of the soil colloids in the 1:10 suspension. The pH declined, changing from the control with 7.13 to 5.70 in the largest urea application. In the 1:1 extract the specific conductance likewise increased, with progressively larger amounts of urea added, from 5.50 to 30.5 $\times 10^{-4}$ mhos. Except for NH_4 all of the cations progressively increased with larger

TABLE 4

Effect of the biological destruction of increasing amounts of urea upon Yolo loam after an incubation period of 11 weeks

UREA: MGM.-ATOMS OF NITROGEN PER KGM. SOIL	0	4	8	15	30
1:10—suspension:					
Susp'd. solids: mgm. in 20 ml	89.0	73.8	13.2	0.7	−1.3
Extract—soluble solids, do	1.5	2.3	3.0	4.7	7.6
Sp. cond. Mhos $\times 10^{-4}$	1.24	1.72	2.04	2.80	4.41
Quin pH 1:1 suspensions H_2O	7.13	6.85	6.62	6.29	5.70
N KCl	5.94	5.82	5.70	5.52	5.16
Extract: 1:1 = soil:H_2O					
Sp. cond. Mhos $\times 10^{-4}$	5.50	9.24	13.10	19.45	30.5
Ions m.e./kgm.:					
NH_4	0.06	0.16	0.14	0.14	0.14
K	0.24	0.37	0.39	0.54	0.70
Na	0.94	1.04	1.13	1.24	1.39
Ca	1.61	3.04	4.09	6.82	11.32
Mg	2.49	4.66	6.50	10.76	17.24
Total cations	5.34	9.27	12.25	19.50	30.79
HCO_3	0.48	0.32	0.32	0.24	0.16
Cl	0.20	0.20	0.22	0.17	0.20
NO_3	3.58	7.58	10.64	17.20	29.36
SO_4	0.60	0.57	0.50	0.54	0.54
PO_4	0.05	0.04	0.03	0.05	0.04
Total anions	4.91	8.71	11.71	18.20	30.30

applications of urea. With this particular soil in each 1:1 extract the concentration of Mg ions was higher than that of any other cation.

It is of interest to note that in these tests the rates of increase of equivalents of Ca and Mg ions was greater than those of the other basic cations, and that the rates of these two were practically equal. The rate of increase of equivalents of NO_3 ions was slightly greater than those of Ca and Mg, although they varied practically together. As would be expected from the nitrification of progressively larger amounts of urea, the NO_3 ions increased, and along with the accompanying decrease in pH, the HCO_3 ions decreased.

SUCROSE TREATMENTS

The absorption of nitrates attending the destruction of sucrose by microorganisms can be considered the reverse process to that involved in the nitrification of urea by which the concentrations of nitrates can be built up in the

TABLE 5

Effect of the biological destruction of sucrose on "nitrated" Fresno fine sandy loam as measured at various times during the incubation

SUCROSE MGM.-ATOMS CARBON per kilo	TIME OF INCUBATION WEEKS	1:10 = SOIL : H_2O RATIO			QUIN pH AT 25°C. 1:1 SUSPENSION		*N* KCl EXTRACT AT pH 1 M.E. PER KILO	
		Mgm. in 20 ml.		Sp. cond.				
		Susp'd solids	Soluble solids	Mhos × 10^{-4}	H_2O	*N* KCl	NH_4	NO_3
0	3/7	2.5	9.8	6.05	3.65	3.48	0.72	41.8*
	2	2.0	12.8	6.48	3.93	3.75	0.50	36.3
	4	0.8	12.9	6.67	4.26	4.09	1.58	37.7
	7	0.8	13.2	6.76	4.33	4.11	1.71	43.90
75	1	7.4	12.4	6.18	4.13	3.86	1.14	34.6
	2	8.5	11.2	6.03	4.47	4.28	2.56	33.3
	4	7.5	10.5	5.56	4.69	4.48	2.97	30.8
	7	3.6	11.3	6.38	4.60	4.52	4.01	41.42
	16	6.4	8.7	6.09	4.93	4.67	5.20	36.76
150	1	12.9	13.4	6.22	4.33	3.97	1.28	36.6
	2	11.0	10.8	5.95	4.63	4.42	2.79	31.9
	4	12.6	9.2	4.78	5.10	4.86	4.66	26.6
	7	6.7	10.3	5.82	4.96	4.89	6.94	38.61
	16	9.3	8.1	5.80	5.13	4.88	6.74	35.64
300	1	17.8	15.4	5.87	4.26	4.01	1.04	38.7
	2	18.8	9.0	5.25	5.29	5.08	2.68	28.4
	4	18.5	6.7	3.98	6.07	5.80	7.32	21.7
	7	12.2	8.8	5.33	5.46	5.30	9.14	33.35
	16	14.6	7.0	5.29	5.63	5.33	8.88	31.00
750	2	14.0	13.2	4.98	4.85	4.52	3.60	25.2
	4	20.5	6.5	3.24	7.23	6.99	10.34	12.34
	7	17.4	6.5	4.40	6.00	5.83	14.27	26.26

* See footnote to table 1.

soil. We shall accordingly consider the indirect or dynamic effects of this reverse process upon the same soils.

The effects of the biological destruction of sucrose on "nitrated" Fresno fine sandy loam, as determined in these experiments at various times during the incubation period, are given in table 5. At all times during incubation there was increased dispersion with larger additions of sucrose. With the larger

applications, the amounts of suspended solids seemed to reach a maximum at about 4 weeks, the amount of dispersion diminishing after that time. The highest dispersion was obtained in the largest rate of sucrose treatment at 4 weeks of incubation. The amount, 20.5 mgm., was slightly more than 75 per cent of that obtained from the untreated control of the Fresno fine sandy loam, in the urea treatments of table 1. Here sucrose was able nearly to overcome the effects of the "nitrating" treatment given this soil.

TABLE 6

The effect of the biological destruction of increasing amounts of sucrose on "nitrated" Fresno fine sandy loam after 4 weeks of incubation

SUCROSE: MGM.-ATOMS OF CARBON PER KGM. SOIL	0	75	150	300	750
1:10—suspension:					
Susp'd. solids mgm. in 20 ml	0.8	7.4	12.6	18.4	20.6
Extract—soluble solids	12.9	10.5	9.2	6.7	6.4
Sp. cond. Mhos $\times 10^{-4}$	6.67	5.56	4.78	3.98	3.24
Quin pH 1:1 suspensions H_2O	4.26	4.69	5.10	6.07	7.23
N KCl	4.09	4.48	4.86	5.80	6.99
Extract 1:1 = soil: H_2O					
Sp. cond. Mhos $\times 10^{-4}$	47.7	43.3	35.7	29.6	19.6
Ions—m.e./kgm. soil:					
NH_4	1.08	1.82	2.90	4.53	6.34
K	2.92	2.84	2.20	2.60	2.16
Na	1.65	1.36	1.30	1.36	1.39
Ca	30.72	26.44	20.02	13.93	5.83
Mg	12.23	10.52	8.32	6.14	3.93
Total cations	48.60	42.98	34.74	28.56	19.65
HCO_3	0.05	0.16	0.31	1.02	8.50
Cl	0.73	0.73	0.67	0.76	0.64
NO_3	46.50	39.14	31.68	25.88	9.80
SO_4	0.93	0.77	0.61	0.47	0.19
PO_4	1.51	1.44	1.35	0.97	0.57
Total anions	49.72	42.24	34.62	29.10	19.70

As the suspended solids increased, the specific conductance of the extract diminished to a minimum at about 4 weeks. This point also coincided with the time of the lowest amount of nitrates in each of the treatments. With the higher applications of sucrose, as the amount of nitrates again grew, the specific conductance increased and the pH decreased.

By comparing the first columns of Tables 2 and 6 the effect of "nitrating" the Fresno soil on the 1:1 extract may be noted. As should be expected, the concentration of basic cations increased greatly, bicarbonates decreased, and

nitrates increased markedly. Sulfates increased slightly, and soluble phosphates increased about 150 per cent.

The effects of the sucrose treatments on "nitrated" Fresno fine sandy loam after an incubation period of 4 weeks are shown more completely in table 6.

TABLE 7

Effect of the biological destruction of sucrose on "nitrated" Yolo loam as measured at various times during the incubation

SUCROSE MGM.-ATOMS CARBON PER KILO	TIME OF INCUBATION WEEKS	1:10 = SOIL: H_2O RATIO			QUIN pH AT 25°C. 1:1 SUSPENSION		*N* KCl EXTRACT AT pH 1 M.E. PER KILO	
		Mgm. in 20 ml.		Sp. cond. Mhos × 10^{-4}				
		Susp'd solids	Soluble solids		H_2O	*N* KCl	NH_4	NO_3
0	1	.....	7.8	3.88	6.00	5.45	0.94	25.0*
	4	1.1	5.1	3.78	6.11	5.47	1.64	22.3
	11	−0.4	5.8	4.52	6.07	5.28	1.00	29.44
50	1	0.9	7.2	3.54	6.21	5.58	0.99	22.2
	4	1.3	4.8	3.52	6.24	5.47	1.56	25.28
	11	0.3	5.3	3.92	6.09	5.37	0.64	25.22
100	1	4.5	6.7	3.22	6.40	5.70	1.58	20.4
	2	3.9	5.3	3.15	6.50	5.71	2.28	19.56
	4	3.7	4.2	3.31	6.29	5.58	2.24	19.52
	7	4.7	3.8	3.22	6.41	5.60	2.20	18.62
	11	0.8	4.8	3.52	6.12	5.40	0.76	22.00
200	1	15.2	6.4	2.96	6.71	5.99	2.04	16.9
	2	11.1	5.0	2.64	6.80	5.99	2.96	15.66
	4	8.6	4.1	2.90	6.58	5.80	3.68	16.24
	7	7.9	3.3	2.94	6.57	5.70	2.72	16.62
	11	1.5	4.5	3.33	6.19	5.42	0.52	20.62
400	1	17.8	6.8	2.86	6.97	6.21	2.34	13.5
	2	30.6	4.4	2.32	7.32	6.41	4.82	11.74
	4	27.6	3.6	2.50	7.04	6.17	5.84	11.48
	7	17.2	3.2	2.57	6.80	5.89	3.24	13.94
	11	5.7	3.9	2.84	6.45	5.70	2.00	16.60
750	2	27.1	6.1	2.42	7.29	6.48	3.02	9.42
	4	117.3	3.2	1.84	7.51	6.63	7.60	3.80
	7	49.2	3.0	2.22	7.25	6.31	4.24	9.80

* See footnote to table 1.

These analyses were made at the time of the highest amount of suspended solids where the largest amount of sucrose had been added. Coincident with this high dispersion, the specific conductance of the extract was at a minimum. The *N* KCl extract at pH 1 showed a high amount of ammonia, which was also reflected by appreciable amounts in the water extract.

In the 1:1 extract the concentration of NH_4 ions rose from 1.08 m.e. in the control to 6.34 in the case of the largest sucrose treatment. Potassium and Na ions in general declined, though some irregularities occurred. Calcium and Mg ions diminished progressively, the change of the former being much more marked than that of the latter. Among the anions, the HCO_3 ions increased along with the pH of the suspensions. The NO_3, SO_4, and PO_4 ions diminished progressively and materially. The Cl ions varied slightly in concentration.

The number of the equivalents of the Ca, Mg, and NO_3 ions, which were those of greatest numerical fluctuation, changed at about the same rate with increased sucrose treatment. Potassium and Na ions, as was found also in the nitrification experiments, changed less abruptly. With this soil, as the amount of sucrose increased, the concentration of PO_4 ions in the 1:1 extract decreased. Although some of this may have been due to a chemical precipitation following the higher pH values attained, still a considerable amount of the disappearance of the phosphates may have been due to absorption by microorganisms. In this connection, Thompson *et al.* (42) have shown phosphate diminution in soil cultures of *Azotobacter chroccoccum* when dextrose was added.

As with the urea treatments, the Yolo loam gave a picture somewhat different from that of the Fresno soil with respect to the results of the sucrose treatments. The effects of the biological destruction of sucrose upon the "nitrated" Yolo soil are given in table 7. With regard to suspended solids, the highest amount of sucrose added after 4 weeks of incubation caused the dispersion of the colloids, so that 117.3 mgm. remained in suspension after 24 hours. This was higher than the Yolo loam (cf. urea control table 3) had at first, namely, after 1 week's incubation.

With the treatment of 100 m.a. of carbon as sucrose, and larger applications also, the pH rose to a maximum and subsequently declined. In each case, this maximum pH occurred at the same time as the lowest specific conductance, and in the case of the two largest applications of sucrose, the maximum pH was coincident with the smallest amount of nitrate and with fairly large, if not the largest, amount of ammonia.

Of the more complete analyses, one set was made after 4 weeks of incubation and the other after 11 weeks. The one after 4 weeks is shown in table 8. Here the electrolytes were withdrawn sufficiently in the 750 m.a. of carbon added as sucrose to disperse the soil colloids to the highest value obtained in all the tests. The sucrose treatment had been able to overcome completely the flocculating effects of the HNO_3 added. Undoubtedly, this was aided by the higher pH obtained with the NH_3 liberated from the decomposition of the microbial protein. Potassium ions decreased except in the case of the 100 m.a. treatment, and Na ions progressively decreased with added sucrose, also. Calcium and Mg ions diminished markedly. Among the anions, HCO_3 ions increased materially, and the concentration of the chloride ions rose also. Sulfate ions decreased progressively, but PO_4 ions showed no consistent action.

Between the fourth week (cf. table 8) and the eleventh week (cf. table 9) the nitrification of some of the microbial proteins and similar compounds previously formed, occurred as shown by the considerable increase in nitrates during that time. Potassium and Na ions showed some differences, but they were hardly consistent with any easy explanation. With the larger sucrose applications Ca and Mg ions rose in concentration in the period following the

TABLE 8

Effect of the biological destruction of increasing amounts of sucrose upon "nitrated" Yolo loam after 4 weeks of incubation

SUCROSE: MGM.-ATOMS OF CARBON PER KGM. SOIL	0	50	100	200	400	750
1:10—suspension:						
Susp'd solids mgm. in 20 ml	1.1	1.4	3.7	8.6	27.6	117.3
Extract soluble solids	5.1	4.8	4.2	4.1	3.6	3.2
Sp. cond. Mhos $\times 10^{-4}$	3.78	3.52	3.31	2.90	2.50	1.84
Quin pH 1:1 suspensions H_2O	6.11	6.24	6.29	6.58	7.04	7.51
N KCl	5.47	5.47	5.58	5.80	6.17	6.63
Extract 1:1 = soil:H_2O:						
Sp. cond. Mhos $\times 10^{-4}$	28.60	24.45	24.20	17.88	15.41	6.65
Ions m.e./kgm. soil:						
NH_4	0.48	.....	0.44	0.56	0.72	0.81
K	0.77	.....	0.93	0.57	0.61	0.36
Na	1.50	.....	1.42	1.26	1.21	1.01
Ca	9.82	.....	8.65	5.52	4.51	1.90
Mg	14.96	.....	11.86	8.68	7.23	3.16
Total cations	27.53		23.30	16.59	14.28	7.24
HCO_3	0.24	0.34	0.40	0.52	1.24	3.96
Cl	0.22	0.22	0.28	0.34	0.45	0.73
NO_3	26.10	.....	21.60	14.80	11.90	2.60
SO_4	0.40	.....	0.30	0.21	0.13	0.09
PO_4	0.04	.....	0.02	0.04	0.03	0.06
Total anions	27.00	.....	22.60	15.91	13.75	7.44
SiO_2 p.p.m.	58	.....	50	40	35	22

first weeks of incubation. Among the anions, the bicarbonates diminished, whereas sulfates and nitrates increased during this period.

It it interesting to note that as nitrates diminished, presumably to form proteins in the microorganisms, sulfates also declined in amount (cf. table 8). These may be presumed to have also gone into the formation of proteins. The increases of both nitrates and sulfates between the fourth and eleventh weeks was presumably brought about by the decomposition of proteins previously formed. Again the equivalents of Ca, Mg, and NO_3 ions responded to

change at about the same relative rate, whereas K and Na ions reacted much more slowly.

Results similar to those reported herein, with the possible exception of those for SO_4 ions, were obtained by Stewart (41) and by Burd and Martin (11, 12) in studying the effect of the growth of higher plants on soils. This similarity

TABLE 9

Effect of the biological destruction of increasing amounts of sucrose upon "nitrated" Yolo loam after 11 weeks of incubation

SUCROSE: MGM.-ATOMS OF CARBON PER KGM. SOIL	0	50	100	200	400
1:10 suspension:					
Susp'd solids mgm. in 20 ml	−0.4	0.3	0.8	1.6	5.7
Extract—soluble solids	5.8	5.3	4.8	4.5	3.9
Sp. cond. Mhos × 10^{-4}	4.52	3.92	3.52	3.33	2.84
Quin. pH 1:1 suspensions H_2O	6.07	6.09	6.12	6.19	6.45
N KCl	5.28	5.37	5.40	5.42	5.70
Extract—1:1 = soil:H_2O:					
Sp. cond. Mhos × 10^{-4}	31.42	27.21	25.78	24.22	20.21
Ions m.e./kgm. soil:					
NH_4	0.15	0.10	0.06	0.12	0.30
K	0.76	0.69	0.61	0.61	0.52
Na	1.46	1.37	1.38	1.44	1.38
Ca	12.60	12.19	9.00	8.55	6.45
Mg	15.52	12.53	11.66	11.26	9.88
Total cations	30.49	26.88	22.71	21.98	18.53
HCO_3	0.36	0.36	0.36	0.40	0.55
Cl	0.20	0.20	0.14	0.14	0.14
NO_3	29.60	25.40	23.50	22.34	17.78
SO_4	0.49	0.39	0.35	0.29	0.24
PO_4	0.07	0.06	0.06	0.06	0.06
Total anions	30.72	26.41	24.41	23.23	18.77
SiO_2 p.p.m.	56	54	56	51	46

of behavior was not unexpected in view of the fact that it was the growth of minute plants deriving their energy from the sucrose added which was responsible for the changes.

DISCUSSION

Biochemical and cation—exchange reactions

Urea in its decomposition goes through the following reaction:

$$(NH_2)_2CO + 2\ H_2O = (NH_4)_2CO_3$$

The $(NH_4)_2CO_3$ in solution partially reacts with the base exchange complex, and the pH of the medium goes up. The ammonium carbonate is nitrified according to the following reaction:

$$(NH_4)_2CO_3 + 4O_2 = 2HNO_3 + CO_2 + 3H_2O$$

As the ammonium carbonate is oxidized to nitric acid, the hydrogen of the acid is dissociated as H ions. Brioux (9), Pierre (33), Jones (25) and the experiments reported herein showed the effect of urea decomposition on the reaction of soil; first the pH was raised, then it decreased to a value below that of the initial pH. Meanwhile, the H ions thus formed reacted with the replaceable cations of the exchange complex, giving a lower pH of the suspension and a higher specific conductance of the extract, and increasing concentrations of K, Na, Ca, and Mg ions in the soil solution. The theory connected with this phase of the problem has been explained in considerable detail by such workers as Pierre (33) and F. E. Allison (1).

With the exception of urea, most organic nitrogenous substances which are initially concerned in ammonification and nitrification are proteinaceous in nature. Pure proteins (essentially neutral in reaction) contain only carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus. Any protein, on being fully oxidized with oxygen and its products dissolved in water, will yield 1 mole each of H_2CO_3, HNO_3, H_2SO_4, and H_3PO_4 for each gram-atom respectively of carbon, nitrogen, sulfur, and phosphorus contained in the original protein. The effect of the H_2CO_3 as an acid is variable, depending upon the properties of the system being considered. At low pH values the H_2CO_3 is easily lost from the soil as CO_2, and that which remains is but weakly dissociated to form H ions. With increasing alkalinity its acidifying effect becomes more pronounced, but is here considered almost negligible for neutral and acid soils. With the exception of carbon, there are many more gram-atoms of nitrogen than of the other elements named in each of the proteins. Consequently, the nitrification of organic materials to form nitrates may be expected to dominate in soils where H_2CO_3 has but little effect as an acid. Thus, we would expect results similar to those obtained with urea from other nitrogen-containing organic materials on oxidation to nitrates, with due allowance for the chemical action of the H_2CO_3, H_2SO_4, and H_3PO_4 simultaneously produced. These acids must result from the complete oxidation of these organic materials whether the processes take place by purely chemical means in the laboratory or biologically in the soil. The writer has developed this theory more fully elsewhere (14).

With larger applications of sucrose, undoubtedly the most important contributing cause of the increase in pH was what is known to plant physiologists as "physiological alkalinity" currently explained by a more rapid absorption of NO_3 ions than of the basic cations in the soil solution, but more clearly explained by Pantanelli (32) and also more recently by F. E. Allison (1) by the absorption of HNO_3 out of the soil solution. Pantanelli postulated the absorption of H

ions with the NO_3 ions leaving the OH ions with the cations with which the NO_3 ions were originally paired. In studies involving the absorption of ions with seedling plants, Hoagland (21) suggested the replacement of NO_3 ions with HCO_3 ions by the plants. Practically, in a system in which CO_2 was present in sufficient amounts, the results would not be different from those proposed by Pantanelli and Allison as

$$OH^- + CO_2 = HCO_3^-$$

If the soil organisms absorb nitrates largely as HNO_3, we would expect the reversal of the conditions we found with the nitrification of urea. As HNO_3 was formed from urea, a large part of the H ions thus made available replaced the basic cations in the exchange complex. With the removal of HNO_3 by the microorganisms the basic cations went back into the places formerly occupied by them.

Work already cited suggests the absorption of nitrates as HNO_3. It can be shown even more definitely by a line of theoretical reasoning that it is in the form of HNO_3 that plants use nitrates in producing pure proteins. As stated in the foregoing, pure proteins, on complete oxidation with oxygen give 1 mole each of H_2CO_3, HNO_3, H_2SO_4, and H_3PO_4 for each gram-atom of carbon, nitrogen, sulfur, and phosphorus, respectively, contained in them. In the production of the same pure proteins, plants must, therefore, use 1 mole each of H_2CO_3 (or CO_2), HNO_3, H_2SO_4, and H_3PO_4 for each gram-atom of carbon, nitrogen, sulfur, and phosphorus[3] respectively, occurring in the proteins thus formed where these elements are furnished as bicarbonates (or CO_2), nitrates, sulfates, and phosphates. Other nitrogenous organic compounds occur in plants. But as proteins are the dominant ones, their formation is undoubtedly the dominant one in the utilization of nitrates by plants. It is entirely probable that with the production of many other organic compounds containing carbon, nitrogen, sulfur, and phosphorus in plants (both higher and lower forms), H_2CO_3 (or CO_2), HNO_3, H_2SO_4[4], and H_3PO_4 are the fully oxidized forms of the respective elements which are used.

Nitrates decreased in the soil solution when sucrose was added in our experiments. It might be argued that the basic cations, occurring in the soil solution with them were absorbed by the microorganisms at the same time. It is true that a neutral salt added to a soil will lower the pH, and that the withdrawal of a neutral salt should likewise increase the pH. With the Fresno soil (cf. control table 1), at 3 days of incubation, the water suspension gave a pH of 7.33, whereas the *N* KCl suspension gave a pH of 6.36, a pH change of approximately 1 unit. At other times, the pH change with KCl added was much less. Here the addition was of 1,000 m.e. of KCl per kgm. In the case of the

[3] Phosphorus, according to Haas and Hill (18), probably does not occur in plant proteins, but is here included as a theoretical possibility.

[4] Generally only two of these three fully oxidized forms would be utilized in the formation of any one substance by a plant not capable of photosynthesis.

"nitrated" Fresno soil, the withdrawal of nitrates was a little less than 40 m.e. per kgm. This amount of withdrawal is far too small to account for a pH change of 3 units.

Unless we assume a marked alteration in the exchange complex of this soil, we must admit that H ions left it and basic cations entered it. Otherwise, there would have been no such change in the pH of the 1:1 aqueous suspension. It seems, therefore, probable that a very considerable part of the basic cations disappearing from the soil solution re-entered the exchange complex as predicated by F. E. Allison (1).

It is recognized that when organic materials are broken down to purely inorganic compounds, the cations which appear in the soil solution do not come from the exchange complex alone. Undoubtedly, a considerable proportion of these was present within the organic materials at the start of its decomposition. Similarly, all of the cations which disappeared from the soil solution due to the withdrawal of H ions did not enter the exchange complex. Some undoubtedly were absorbed by the organisms themselves.

Another biological factor causing a change in the pH was an increasing amount of NH_4 ions among the replaceable cations as disclosed by the *N* KCl extract at pH 1. Especially with the Fresno soil, a soil in which nitrification was delayed in the urea treatments, a considerable amount of NH_4 ions accumulated. As, obviously, the principal source of the ammonia was the nitrate occurring in the soil at the beginning, the transformation from NO_3 to NH_4 ions, or rather the change from HNO_3 to NH_4OH, was the cause of the increase in pH as far as this factor is concerned.

Although the chain of processes by which NH_4 ions increased in the exchange complex was of small moment in determining the exact pH at any one time, it is necessary to know the processes by which the ions arose, in order to appreciate what to expect with a continuation of them. With an increase of NH_4 ions before the NO_3 ions stopped decreasing, the question comes up as to the direct origin of the ammonia. Was it formed directly from the nitrate or was it formed indirectly by deaminization of amino compounds which in turn had been formed from the nitrates? Kostytschew and Tswetkowa (30) showed that ammonia is one of the stages between nitrates and amino compounds.

In our experiments (cf. tables 5 and 7), ammonia certainly began to appear at the end of 1 week, whereas in the larger rates of sucrose application the lowest point of nitrate concentration was not reached until the fourth week. This sequence of events did not preclude the possibility of ammonia having arisen by the deaminization of microbial proteins and similar compounds already formed from the original nitrates. It is natural to suppose that this was the dominant process after the nitrates began to increase. It could very well have been the dominant process also as nitrates were diminishing, simply by the deaminization of compounds (in the old microbial cells) already formed. Some other evidence is applicable here. Jensen (24) showed that dried fungous bodies as well as dried growth of *Actinomyces*, when added to sand, produced

considerable quantities of ammonia in 10 days. Our data give no inkling as to whether some of the ammonia first appearing was directly formed from nitrate or secondarily from amino compounds.

A third factor was operative in the change of pH. The control "nitrated" soils which received no sucrose (cf. tables 5 and 7) gradually increased in pH values during the course of incubation. This is especially marked with the Fresno soil (cf. table 5) where the pH in the aqueous suspensions increased from 3.65 after 3 days to 4.33 after 7 weeks of incubation. This increase might have been due to a slow exchange of basic cations of the inner layers of the soil colloidal crystals by the H ions from the soil solution or from those of the outer layers of the soil colloids, namely, that part referred to as the "exchange complex." It might have been due, of course, to a change to a weaker kind of alumino-silicic acid, i.e., to a molecular rearrangement where H ions would be less dissociated than formerly.

Resultant effects on colloid dispersion

Factors which undoubtedly cause changes in colloid dispersion in the soils used in these experiments include:

Variations in the composition and concentration of the electrolytes in the dispersion medium. Most of the changes in colloid dispersion which are not due to experimental error are attributed to this factor.

Variation in the amounts of each of the different cations in the exchange complex. The influence of the variation in the amount of H ions in the exchange complex on colloid dispersion is indicated by the work of Bradfield (7). He added various mixtures of KCl and KOH and found over 10 times as much electrolyte (as measured by the specific conductance of the dispersion medium) was required to flocculate his soil colloids at pH 8 as at pH 5.2. The dispersing action of ammonia on soil colloids follows the same general principle.

The deflocculating effect of ammonia formed through biological agencies in soils observed in these experiments (cf. table 1) has been noted elsewhere. Russell (36) warned against the use of liquid manure, especially in excessive amounts, on heavy soils because of the resulting deflocculation of the soil colloids and the formation of a crust on the soil surface in the field. Hager (19) has explained this deflocculation as due to $(NH_4)_2CO_3$ formed from the decomposition of the nitrogenous compounds in the liquid manure. On soils which nitrify ammonia readily, he has stated that this unfavorable condition soon changes as the nitrates are formed from the ammonia.

Subject only to the ability of a soil, the colloids of which remain electronegative, to form and retain nitrates from an adequate supply of organic materials, the soil solution must become concentrated enough with continual formation of these compounds to precipitate the colloids in a 1:10 aqueous suspension. This is what we find with the Yolo soil. If the Fresno soil had been able to nitrify urea completely with the higher concentrations of urea tried, this would have resulted in flocculation of the colloids, as the preliminary experiments not reported here showed that HNO_3 of equivalent concentration

would do. This was also shown by the control "nitrated" Fresno soil in the sucrose treatments reported herein.

Given a system in which HNO_3 artificially or biologically added causes flocculation of the soil colloids, it would seem natural that the effect of biological processes which would result in the withdrawal of HNO_3 from the soil solution by microorganisms which are supplied with sucrose and similar materials would cause the dispersion of the soil colloids if continued long enough, unless by-products of these processes would keep them flocculated. In this regard, the question has been raised as to the possible effect of CO_2 liberated by the decomposition of the sucrose. Judged by its dissociation constant, H_2CO_3 is but slightly effective as an acid below a pH of 6, and with decreasing pH values its effectiveness becomes even less, so that at the pH of these experiments it would not be expected to exert much influence, especially when CO_2 has an opportunity to escape. If these tests were conducted with soils at a higher pH, we might expect the H_2CO_3 liberated to keep the soils flocculated. Thus, Breazeale (8) described an experiment where the suspension of a soil containing $CaCO_3$ was flocculated by bubbling CO_2 through it. At a pH of 8 or more H_2CO_3 has an appreciably acid effect.

GENERAL DISCUSSION

The water-soluble substance, urea, produced changes in colloid dispersion correlated with the HNO_3 produced by the organisms concerned, whereas the water-soluble sucrose produced changes correlated with the HNO_3 withdrawn by the organisms.

In a like manner, applications of non water-soluble organic materials would be expected to produce like results in proportion to the nitrates produced or nitrates consumed by the organisms breaking them down, except in so far as these non water-soluble materials would influence the properties of the soil directly or statically.

In general, the chemical as well as the physical effects on soils here shown are best interpreted in a broad way, not on the basis of nitrates produced or consumed, but rather on the basis of the H ions appearing and disappearing in the soil system. Thus it was the H ions that became completely ionized as the nitric acid was produced from urea which were the active agents of the changes which followed. On the other hand, it was the withdrawal of H ions with the NO_3 ions to form microbial proteins which carried these chemical and physical processes through in the opposite direction where sucrose and other materials inducing nitrate-disappearance were added.

Other experiments have shown the effects of H ions added or biologically produced upon the physical properties of soils. Stephenson and Powers (40) obtained increased flocculation of the soil colloids with larger applications of sulfur, which, as a result of its biological oxidation, yielded a large amount of H ions with the SO_4 ions produced. Haynes (20) obtained an increased rate of percolation of water through black alkali soils after sulfur applications.

Kelley and his co-workers (28, 29) found improved penetration of irrigation water and a general improvement in tilth where sulfur, iron sulfate, and alum were applied in sufficient quantities to black-alkali soils. All three of these treatments caused H ions to increase in the soil.

Practices resulting in the increase of electrolytes in the soil, especially those furnishing divalent cations, have in many cases markedly improved the tilth of the soil. It would be expected that biological activities which would result in increased H-ion concentrations in soils whose exchange complex is well supplied with divalent cations would increase the latter in the soil solution, which in turn would cause a better physical condition of the soil. However, the experience of Sievers and Holtz (38) that the value of organic matter in improving the physical condition of the soil is proportional to its nitrogen content is best explained, not on the basis of the static rôle of the organic materials, but rather in part at least upon the amount of nitrates (therefore H ions) produced in its decay. The same explanation, which takes into account the possibility that the growth of higher plants may also be a means of reducing H ions by the absorption of nitrates, is suggested for Chapman's findings. Stephenson's procedure is so nearly like our own that in so far as our results check his, the same explanations are given for them. The explanation of his other observations must await further experimentation.

One purpose of this paper has been to draw attention to the purely dynamic rôle of organic substances in soils. The methods of procedure used and the water-soluble substances selected were such as to emphasize this function particularly. The results herein reported cannot, we believe, be explained on the basis of "static" organic matter. Until the dynamic rôle is definitely recognized, then, it cannot be differentiated from the static one. Though some of the functions of organic matter which have heretofore been ascribed to its static rôle may later be credited to its dynamic one, it is the opinion of the writer that both should be recognized.

SUMMARY

Organic materials may influence the physical condition of soils in two ways: *directly* by their purely physical properties, and *indirectly* by changes in the electrolyte concentration of the soil solution resulting from the utilization of these materials by microorganisms. The purpose of this paper is to present a study of this *indirect* effect.

The organic materials used were urea and sucrose—both non-colloidal, water-soluble, and practically free from electrolytic properties, and therefore of negligible direct effect on the soil colloids, either from a purely physical point of view or from a physico-chemical one.

The criterion of colloid dispersion was the amount of suspended solids (in a soil suspension whose soil-water ratio was generally 1 to 10) obtained according to a standardized procedure.

Ammonification of urea proceeded normally in both soils. Incomplete

nitrification of the largest applications of urea to one soil, Fresno fine sandy loam, resulted in large accumulations of ammonia, and in increases in pH and colloid dispersion. These results are attributed to the $(NH_4)_2CO_3$ arising from ammonification of the urea. Normal nitrification of urea in the other soil, Yolo loam, caused decreases in pH and colloid dispersion. It also increased specific conductance and concentrations of nitrates and basic cations, especially Ca and Mg in the liquid phase. These effects are assignable to the HNO_3 produced in the nitrification of the urea.

The application of sucrose to "nitrated" (the nitrates had previously been increased by the addition of HNO_3) samples of both soils caused decreases in specific conductance, in cations, and in nitrate ions of the liquid phase, and increases in pH and colloid dispersion. These findings had their origin in the withdrawal of HNO_3 from the liquid phase by microorganisms to produce microbial proteins. The basic cations moved meanwhile to the exchange complex to take the places formerly occupied by the H ions.

In the sucrose-treated soils NH_4 ions began to increase early in the incubation period. Later, especially in the Yolo soil, nitrates, basic cations, and specific conductance increased in the liquid phase, while pH and colloid dispersion in general decreased. We account for these facts by the breakdown of the microbial proteins formed, and the conversion of the nitrogen first to ammonium carbonate and subsequently to HNO_3—a reversal of the processes taking place early in the incubation of the sucrose.

During incubation the "nitrated" control (no additions of sucrose) samples slowly increased in pH, presumably in adjustment to the HNO_3 added in "nitrating."

Other investigators have shown that some of the same effects observed in this study, namely, decreased dispersion and increased rate of percolation accompanying the increase of nitrates, have also been obtained with other H-ion producing amendments (sulfur, iron sulfate, alum, etc.). Often marked improvements in soil tilth were noted in the field as results of these amendments. May the improvement in soil tilth following the application of some organic materials not in part result from the formation of acids (HNO_3, H_2SO_4, H_2CO_3, H_3PO_4, etc.) arising from the utilization of these organic substances by microorganisms?

REFERENCES

(1) ALLISON, F. E. 1931 The comparative effects of concentrated nitrogenous fertilizers on permanent soil acidity. *Jour. Amer. Soc. Agron.* 23: 878–908.

(2) ALLISON, R. V. 1926 The influence of plant growth upon the state of aggregation of soil particles. *Actes IV ème Conf. Internat'l. Pédologie* (1924) 3: 578–597.

(3) AMES, J. W. 1921 Solvent action of nitrification and sulfofication. *Ohio Agr. Exp. Sta. Bul.* 351: 223–257.

(4) BAVER, L. D. 1930 The effect of organic matter upon several physical properties of soils. *Jour. Amer. Soc. Agron.* 22: 703–708.

(5) BOUYOUCOS, G. J. 1932 Studies on the dispersion procedure used in the hydrometer method for making mechanical analysis of soils. *Soil Sci.* 33: 21–26.

(6) BRADFIELD, R. 1931 Some chemical reactions of colloidal clay. *Jour. Phys. Chem.* 35: 360–373.

(7) BRADFIELD, R. 1923 The relation of hydrogen-ion concentration to the flocculation of a colloidal clay. *Jour. Amer. Chem. Soc.* 45: 1243–1250.

(8) BREAZEALE, J. F. 1924 The injurious after-effects of sorghum. *Jour. Amer. Soc. Agron.* 16: 689–700.

(9) BRIOUX, CH. 1924 Influence de l'urée employée comme engrais sur le réaction du sol. *Compt. Rend. Acad. Sci.* (Paris) 179: 915–917.

(10) BURD, J. S. 1925 Relation of biological processes to cation concentrations in soils. *Soil Sci.* 20: 269–283.

(11) BURD, J. S., AND MARTIN, J. C. 1924 Secular and seasonal changes in the soil solution. *Soil Sci.* 18: 151–167.

(12) BURD, J. S., AND MARTIN, J. C. 1931 Secular and seasonal changes in soils. *Hilgardia* 5: 455–509.

(13) CHAPMAN, J. E. 1928 The effects of organic matter on the tillage of a clay soil. *First Internatl. Cong. Soil Sci.* (1927) 1: 443–445.

(14) CONRAD, J. P. 1933 Acidity and alkalinity produced by changes in the nitrogen, sulfur, and carbon cycles. *Plant Physiol.* 8: 509–524.

(15) DORYLAND, C. J. T. 1916 The influence of energy material upon the relation of soil microörganisms to soluble plant food. *N. D. Agr. Exp. Sta. Bul.* 116: 319–401.

(16) GANGOPADHYAY, R. 1932 Agriculture and Agriculturists of Ancient India. N. A. Mukherjee & Co., Serampore.

(17) GEILMAN, W., AND VAN HAUTEN 1921 Die Änderung der löslichen Bodensalze und der Schlamkurve gedüngter Parzellen im Laufe der Entwicklung von Rüben. *Jour. Landw.* 69: 105–130.

(18) HAAS, P., AND HILL, T. G. 1928 Chemistry of Plant Products, v. 1, p. 427–428. Ed. 4. Longmans, London.

(19) HAGER, G. 1931 Die Kolloidbestandteile des Bodens und die Methoden ihrer Erkennung. *Handb. der Bodenlehre* 7: 45–112, J. Springer, Berlin.

(20) HAYNES, J. D. 1928 Studies with sulfur for improving permeability of alkali soil. *Soil Sci.* 25: 443–446.

(21) HOAGLAND, D. R. 1923 The absorption of ions by plants. *Soil Sci.* 16: 225–246.

(22) HOAGLAND, D. R., AND MARTIN, J. C. 1920 Effect of season and crop growth on the physical state of the soil. Jour. Agr. Res. 20: 397–404.

(23) HUTCHINSON, H. B. 1918 The influence of plant residues on nitrogen fixation and on losses of nitrates in the soil. *Jour. Agr. Sci.* 9: 92–111.

(24) JENSEN, H. L. 1932 The microbiology of farmyard manure decomposition in soil. III. Decomposition of the cells of microörganisms. *Jour. Agr. Sci.* 22: 1–25.

(25) JONES, H. W. 1932 Some transformations of urea and their resultant effects on the soil. *Soil Sci.* 34: 281–299.

(26) KEEN, B. A. 1931 The Physical Properties of the Soil. Longmans, London.

(27) KELLEY, W. P. 1918 Effect of nitrifying bacteria on the solubility of tricalcium phosphate. *Jour. Agr. Res.* 12: 671–683.

(28) KELLEY, W. P., AND ARANY, A. 1928 The chemical effects of gypsum, sulfur, iron sulfate, and alum on alkali soil. *Hilgardia* 3: 393–420.

(29) KELLEY, W. P., AND THOMAS, E. E. 1928 Reclamation of the Fresno type of black-alkali soil. *Calif. Agr. Exp. Sta. Bul.* 455: 1–37.

(30) KOSTYTSCHEW, S., AND TSWETKOWA, E. 1920 Über die Verarbeitung der Nitrate in organische Stickstoffverbindungen durch Schimmelpilze. *Hoppe-Seyler's Ztschr. Physiol. Chem.* 111: 171–200.

(31) KRÜGER, W. 1905 Einfluss der Düngung und des Pflanzenwuchses auf Bodenbeschaffenheit und Bodenerschöpfung. *Landw. Jahrb.* 34: 783–804.

(32) PANTANELLI, E. 1915 Über Ionenaufnahme. *Jahrb. Wiss. Bot.* 56: 689–730.

(33) PIERRE, W. H. 1928 Nitrogenous fertilizers and soil acidity. *Jour. Amer. Soc. Agron.* 20: 254–279.
(34) PURI, A. N., AND KEEN, B. A. 1925 The dispersion of soil in water under various conditions. *Jour. Agr. Sci.* 15: 147–161.
(35) ROBINSON, G. W. 1922 Note on the mechanical analysis of humus soils. *Jour. Agr. Sci.* 12: 287–291.
(36) RUSSELL, E. J. 1932 Soil Conditions and Plant Growth, ed. 6. Longmans, London.
(37) SEELHORST, C. VON, GEILMAN, W., AND HÜBENTHAL, H. 1921 Über den Einfluss von Düngung und Pflanzenwuchs auf die Fallkurve von Wasser-Bodengemischen. *Jour. Landw.* 69: 5–32.
(38) SIEVERS, F. J., AND HOLTZ, H. F. 1926 The significance of nitrogen in soil organic matter relationships. *Wash. Agr. Exp. Sta. Bul.* 206: 1–43.
(39) STEPHENSON, R. E. 1918 The effect of organic matter on soil reaction. *Soil Sci.* 6: 413–439.
(40) STEPHENSON, R. E., AND POWERS, W. L. 1924 Influence of sulfur oxidation on solubility of soil minerals. *Soil Sci.* 18: 317–321.
(41) STEWART, G. R. 1918 Effect of season and crop growth in modifying the soil extract. *Jour. Agr. Res.* 12: 311–368.
(42) THOMPSON, L. G. Jr., SMITH, F. B., AND BROWN, P. E. 1931 Phosphorus assimilation by soil microörganisms. *Soil Sci.* 31: 431–436.
(43) WARINGTON, ROBERT 1900 Lectures on Some of the Physical Properties of Soils. Clarendon Press, Oxford.

THE INFLUENCE OF THE pH OF A CULTURE SOLUTION ON THE RATES OF ABSORPTION OF AMMONIUM AND NITRATE NITROGEN BY THE TOMATO PLANT[1]

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Received for publication June 26, 1933

From the scientific standpoint, the presence of nitrogen in the form of a cation (NH_4^+) in one case, and in the form of an anion (NO_3^-) in the other, leads to theoretical implications which are manifold. From the practical standpoint, the importance of a thorough knowledge of the course of absorption and assimilation of various forms of nitrogen by plants is apparent from a consideration of the huge quantities of nitrogen used in fertilizers every year. Furthermore, the development of synthetic nitrogen fertilizers increases the practical possibilities.

The question of the relative efficiency of NH_4-N and NO_3-N as sources of nitrogen for green plants is one which has often been investigated since the days of Liebig, the great agricultural chemist. Hutchinson and Miller (14, 15), Prianischnikow (33), and Mevius (25) have reviewed the early experiments which proved that plants could absorb and assimilate either NH_4-N or NO_3-N. However, considerable disagreement has been evident concerning the relative efficiency of these two forms of nitrogen, and the controversy apparently is not yet closed.

On the relation of the pH of the culture solution to the absorption of NH_4-N and NO_3-N

It is significant that Maze (22, 23), who used sterile solution culture methods, obtained yields of maize when $(NH_4)_2SO_4$ was used as the source of nitrogen, comparable with those produced when $NaNO_3$ was employed as the source of nitrogen, provided that $CaCO_3$ was present in the culture solutions and that the concentration of $(NH_4)_2SO_4$ did not exceed 0.5 parts in 1,000. The effect of adding the $CaCO_3$ was probably to raise the pH of the solution, an effect which in turn influenced the absorption or assimilation, or both, of NH_4-N by the plants. Maze (23) analyzed the solutions in which his plants had been grown for many days and demonstrated that NH_4-N had been absorbed by the plants.

Pantanelli (30) studied the relative rates of absorption of the respective anions and cations of many salts by placing plants in these single-salt solutions for absorption intervals of 8 hours. Curiously enough, he reported no absorption of NH_4-N by *Vicia faba* from a solution of NH_4NO_3. In contrast, Prianischnikow (33), who used absorption intervals of 2 hours and a solution of NH_4NO_3, reported no absorption of NO_3-N by maize in most cases, but considerable absorption of NH_4-N.

Hoagland (13) presented data showing that the absorption of several anions, including NO_3^-, was greater from a solution of acid reaction (pH 5 to pH 5.5) than from a solution with a neutral reaction (pH 6.8). The plants were grown in the solution for 9 days, after which analyses of the solutions were made.

Theron (43) analyzed solutions in which plants had grown for 3 or 4 days and concluded

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

that the influence of the reaction was most marked on the rates of absorption of the cations. Invariably, more cations were absorbed from alkaline than from acid solutions. He also noted some effect of the reaction of the solutions on the absorption of NO_3-N, but the effect was not so pronounced as that on the cations and was opposite to the effect upon the cations, more anions being absorbed from acid than from alkaline solutions.

Mevius and Engel (26) reported an increased rate of absorption of NH_4-N by maize from a culture solution as the pH of the solution was increased from pH 4.3 to pH 6.1, or from pH 6.1 to pH 7.1.

Sabinin and Kolotova (38) analyzed juices from cut stumps of plants grown in culture solutions and also analyzed the solutions. They concluded that at the acid reaction (pH 4.0 to pH 5.0) anions enter the plant in greater quantities than do cations, and that at the alkaline reaction (pH 7 to pH 8) cations enter more rapidly than the anions.

Niklewski, Krouse, and Lemanczyk (28) worked with single-salt solutions to which a buffer had been added. They noted that the alteration of the pH of the solution affected the absorption of the ions of a salt in a pronounced manner, although the presence of the buffer in some solutions introduced a complication by its effect on the roots of the plants.

Loo (20) allowed corn seedlings to grow for periods of 24 hours in solutions and determined that the amount of NH_4-N absorbed from the solutions increased with an increase in alkalinity and that the amount of NO_3-N absorbed was greatest at a weakly acid reaction.

Naftel (27) grew cotton in culture solutions and placed the plants in fresh solutions for absorption tests of 24 hours. He concluded that NH_4-N was used in larger amounts than NO_3-N by young seedlings until they were from 3 to 5 weeks old, and that after this stage, more NO_3-N was absorbed than NH_4-N. He also observed that NH_4-N absorption increased as the acidity of the culture solution decreased, and that the absorption of NO_3-N was only slightly affected by the reaction of the solution used.

Pirschle and Mengdehl (32) made extensive tests of the absorption of ions by peas, but did not study the effect of the pH of the solutions on the rates of absorption.

Prince, Jones, and Shive (35) studied the absorption of ions by soybeans from a four-salt solution in which the plants were grown. They analyzed the experimental solutions after successive 4-hour absorption intervals and at different stages in the life history of the plants. They found that the plants drew rather heavily upon the NH_4-N during the early stages of growth, and less heavily upon the NO_3-N, but that during the later stages of development, this condition was reversed. Jones and Skinner (17) reported similar data with maize plants and also showed that the concentration of NO_3-N in the culture solution affected the absorption of NO_3-N.

Using an improved technique for testing absorption, Stahl and Shive (41, 42) have shown that not only the age of the plant, but also the type of plant itself influences the rates of absorption of NH_4-N and NO_3-N.

To generalize from the aforementioned experiments: it has been shown that the pH of the solution may affect the rate of absorption of both NH_4-N and NO_3-N by plants, although not all of the investigators have agreed on both of the ions. In most cases, however, it has been observed that a relatively low pH of the culture solution favored a higher rate of absorption of NO_3-N than did a high pH; whereas a relatively high pH of the culture medium resulted in a higher rate of absorption of NH_4-N than did a low pH. Furthermore, it has been shown that when both ions are present in the solution, the relative rates of absorption of the two are influenced by the pH of the medium, by the concentration of the ions in question in the solution, by the age of the plant, and by the species used as indicator plants.

The main purposes of the research described in the following pages were: (*a*) to study the relative rates of absorption of NH_4-N and NO_3-N from a culture solution in which these two forms were present in approximately equal proportions simultaneously; (*b*) to study the influence of the pH of this culture solu-

tion upon the rates of absorption of NH_4-N and NO_3-N by the plant; (*c*) to study the influence of the age of the plant upon these relative absorption rates; and (*d*) to note any possible relationship between these absorption rates and the concentrations of NH_4-N and NO_3-N in the plant tissues. No attempt is here made to consider nitrogen absorption rates as determined systematically by experimental testing in relation to assimilation of nitrogen by the plant, nor in relation to the distribution of the nitrogen fractions in the plant tissues. This feature of the study will be reserved for a later publication.

The tomato plant (*Lycopersicum esculentum*, Mill.) was selected for these investigations because its vegetative development extends over a relatively long period of time during the life cycle, and because it has been shown (44) that its chemical composition is influenced by the pH of the culture solution as well as by the source of nitrogen in the culture medium.

METHODS OF EXPERIMENTATION

Method of growing plants

The seeds, selected[2] from a single plant of the Marglobe variety, were germinated in washed white sand which was kept moist with tap water. One week after germination, the seedlings were removed from the sand, washed free of adhering particles, and placed in a culture solution in 2-quart, colorless glass fruit jars. Three seedlings were fixed in a paraffined cork stopper and suspended in each jar by a method similar to that described by Tottingham (45). White cardboard covers around the culture vessels excluded most of the light from the roots.

The solution used was one of the modified Tottingham solutions described by Jones and Shive (18). Solution $T_3R_2C_2$ of the Jones-Shive series was selected because it contained approximately equal molar concentrations of NH_4-N and NO_3-N and had, in preliminary tests, produced excellent growth of tomatoes. This solution was modified by including only one-third of the amount of KH_2PO_4 called for. The KH_2PO_4 concentration was decreased in order to avoid precipitation, which occurred in the pH 7 solution when the original formula was used. The composition of the modified solution, which had a total osmotic concentration of 0.8 atmosphere, is given in table 1, together with the quantities of acid or alkali added to produce the desired pH.

In addition to the elements listed in table 1, traces of boron, manganese, and iron were added to the solution. Boron in the form of H_3BO_3 and manganese in the form of $MnSO_4 \cdot 2H_2O$ were added in quantities sufficient to give a concentration of 0.5 p.p.m. of each of these elements. Iron in the form of $FeSO_4 \cdot 7H_2O$ was added in quantities necessary to prevent chlorosis of the plants from iron deficiency. Concentrations ranging from 0.5 to 4 p.p.m. were used.

[2] The seeds were obtained from Dr. W. R. Robbins of the New Jersey Agricultural Experiment Station, who had made the selection.

The solution first supplied to the plants was prepared in 5-gallon bottles and adjusted to pH 5.5 with *N* KOH. Only one-half the concentrations given in table 1 were used while the plants were small. The solutions were renewed twice after successive 7-day intervals, once after a succeeding 4-day interval, and once after a final 2-day interval before the plants were placed in solutions of different pH.

After 20 days the plants were transferred from the solution in which they had been previously grown, to the solutions adjusted to pH 4.0, pH 5.0, pH 6.0, and pH 7.0. Five cultures, each including three plants, were grown at each pH level. Nine extra cultures were grown at both pH 4 and pH 7 for transfers to be made later.

At the same time, continuous solution renewal and continuous aeration of all the solutions were started. The method of continuous solution renewal was

TABLE 1

Composition of solutions used

Basic solution

SOLUTION NUMBER	PARTIAL VOLUME-MOLECULAR CONCENTRATIONS			
$T_1R_2C_2$ (modified) Tott.	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$
	.00211	.00292	.00711	.0028

Modifications

QUANTITY	ACID OR ALKALI PER LITER	RESULTING pH
cc.		
0.14	Normal H_2SO_4	4.0
0.08	Normal KOH	5.0
0.84	Normal KOH	6.0
2.25	Normal KOH	7.0

that devised by Shive and Stahl (40), and the method of aeration was that used by Clark and Shive (8) previously described by Allison (1). The importance of continuous solution renewal and continuous aeration of the culture solutions in producing vigorous plants for experimentation can not be overemphasized. Shive and Stahl (40) have mentioned several experiments showing the need of continuous solution renewal. In this experiment, continuous renewal was necessary, not only to maintain a more nearly constant pH, but also to maintain a relatively constant concentration of NH_4-N and NO_3-N in the solutions in replicate culture vessels. The beneficial effects of continuous aeration on root development and top growth in tomatoes in similar solutions have been shown by Clark and Shive (8).

The initial rate of continuous solution renewal was 1 liter in 24 hours. This was increased to 2 liters in 24 hours 5 days before the first absorption test was

made. This rate was further increased to 2 liters in 14 hours 5 days before the second adsorption test was made.

Method of determining absorption rates

The details of the absorption tests will be described in connection with the consideration of the results reported for each test, because certain changes in technique were necessitated with increase in age and consequent size of the plants. The absorption intervals were 6 hours in duration and covered a period of from 9 a.m. to 3 p.m. The three plants included in each culture, together with the cork stopper supporting them, were removed from the culture vessel in which the plants had been growing, the roots of the plants were

TABLE 2

Changes in pH of the culture solution brought about by plants 41 days old

ORIGINAL pH OF SOLUTION IN RESERVOIRS	pH OF SOLUTIONS FROM OUTLETS OF CULTURE VESSELS BEFORE ABSORPTION TEST	FINAL pH OF SOLUTIONS USED FOR ABSORPTION TEST
4.0	4.2–4.4	5.05–5.2
5.0	4.7–4.8	5.3 –5.6
6.0	5.5–5.8	6.0 –6.2
7.0	5.9–6.0	6.2

TABLE 3

Rates of absorption of NH_4-N and NO_3-N by plants 41 days old

pH OF CULTURE SOLUTION	AVERAGE GREEN WEIGHT PER PLANT	N ABSORBED PER 100 GM. GREEN TISSUE IN 6 HOURS		
		NH_4-N	NO_3-N	Total
	gm.	*mgm.*	*mgm.*	*mgm.*
4.0	53.0	3.4	4.8	8.2
5.0	55.1	4.2	5.9	10.1
6.0	64.6	4.6	4.1	8.7
7.0	57.8	6.6	3.0	9.6

rinsed with a very fine stream of distilled water for a definite period of time, and the plants were then placed in the fresh solution in the test jar.

Enough solution was used for the test so that continuous renewal at the same rate previously maintained was possible. This continuous renewal of solution during an absorption test has been shown to be necessary by Stahl and Shive (41). At the end of the test interval the plants were removed from the solution and the roots were again rinsed. This wash water, the solution in the culture vessel, and any solution left in the reservoir of the renewal apparatus, and that in the beaker containing the overflow from the culture vessel, were all collected and diluted to the original volume. Analyses of these solutions showed the amount of NH_4-N and NO_3-N removed from the original solution during the absorption interval. The plants used for the absorption test were returned,

at the expiration of the test interval, to the solutions in which they had been growing, were harvested within a few hours, and were immediately prepared for analyses of the tissues.

The NH_4-N in the test solutions was determined by the method of Sessions and Shive (39) with a modification which involved substituting 0.125 *N* sodium hydroxide for the sodium carbonate originally suggested. The NO_3-N in the test solutions was also determined by the method of Sessions and Shive (39).

Method of determining pH

In making pH determinations of the culture solutions, a series of indicators prepared according to directions given by Clark (9) were used. Brom phenol blue, methyl red, brom cresol green, chlor phenol red, and brom thymol blue were selected because they measured a range of pH values from 3.8 to 7.4. The pH of each solution was estimated to within 0.05 of a pH unit by comparing the color developed by the addition of the required indicator with the colors of a set of LaMotte permanent color standards. The values thus obtained were occasionally checked against those of the same samples determined electrometrically by means of the hydrogen electrode and the type "K" potentiometer.

EXPERIMENTAL RESULTS AND DISCUSSION

Results with plants 41 days old

Experimental procedure.—The first absorption test was made after all the plants had been grown for a period of 14 days in the respective solutions of pH 4.0, 5.0, 6.0, and 7.0 in which they had first been placed. Continuous renewal of the culture solutions had proceeded at a rate of 1 liter in 24 hours until 5 days before the absorption test. At that time the rate of renewal was increased to 2 liters in 24 hours, in order to maintain a more nearly constant pH in the solution in which the plants were growing.

To show the necessity for rapid and continuous renewal of the culture solutions, the data in table 2 are presented. The solutions from the outlets of the culture vessels were collected one-half hour before the plants were transferred to the test solutions. The pH of the sample solution collected is representative of the pH of the solution in the corresponding culture jar, which was well mixed by the aerating system. The final pH of each solution used for the absorption test represents the pH of the solution after it had been diluted to the original volume at the completion of the test.

These data show the marked changes in reaction produced in the culture solutions by the plants, even with continuous renewal proceeding at a rate of 2 liters per day. The table indicates, however, that the changes in pH were rather well defined within narrow limits for replicate cultures grown in any one of the solutions.

Continuous renewal and aeration of the solutions were discontinued for 15 to 60 minutes before the plants were transferred to the fresh solutions for the

absorption tests. One liter of solution having the same composition as that in which the plants had previously been grown was used as the test solution for each culture. Of this solution 450 cc. was placed in a pint jar, in which the roots of the plants were suspended during the absorption interval of 6 hours. The remaining 550 cc. of the solution were dripped through the culture jar during the test interval so that the rate of renewal during the test interval was approximately the same as that preceding the test. It was not practicable to aerate the cultures during the absorption test interval, however. With this exception, the environmental conditions during the absorption test were approximately the same as those preceding the test. Hence, it is reasonable to assume that the rates of absorption of NH_4-N and NO_3-N during the 6-hour test interval must have closely approximated the corresponding rates prevailing just before the plants were transferred to the fresh solutions.

The absorption of NH_4-N and NO_3-N by these plants during the 6-hour interval, as well as the green weights of the plants, are recorded in table 3. The values of the average green weight per plant were calculated from the plants in five replicate cultures, each of which included three plants. Thus each value in the table represents the average for 15 plants. The absorption values are the averages for three of these five cultures, only the three most uniform cultures having been selected for the absorption test. The plants from all five cultures were then harvested and this material was used in the chemical analyses. In order to provide a standard for comparison, the average absorption rates are expressed in milligrams of nitrogen absorbed per 100 gm. of green tissue. The absorption rates per unit of green tissue were calculated separately for each culture, and the averages of the three cultures tested in each solution of the different pH values appear in table 3. To coincide with the aliquots taken for analysis, 100 gm. was chosen as the unit of green plant tissue.

Appearance of plants.—Table 3 shows that those plants grown in the pH 6 solution were the largest at this particular age. Although the differences in average green weight per plant between the plants grown in the pH 6 solution and those grown in the pH 4, pH 5 and pH 7 solutions are sufficiently large to be significant, the differences in average green weight per plant between the plants grown in the solutions at pH 4, pH 5, and pH 7 are probably not sufficiently large to be significant. All the plants were very vigorous and the tops were so much alike that no pronounced differences in character of growth were evident.

From the external appearance of the tops, it is apparent that tomato plants can be grown in a solution including both NH_4-N and NO_3-N over a relatively wide range of pH values without significant differences. But, as will be shown later, the rates of absorption of NH_4-N and NO_3-N by these plants differed remarkably over the pH range used. That is, the external appearance of the plants was quite deceptive, considered from the standpoint of the activity of the plants with respect to nitrogen absorption.

Only the roots showed visible differences in character as a result of the different pH treatments. The roots of plants grown in the pH 4 and pH 5 solutions were very white and had many short laterals, but the roots of plants grown in the pH 6 and pH 7 solutions were not quite so white and had fewer short laterals. The roots in the pH 6 and pH 7 solutions were longer and more abundant, however, than the roots in the pH 4 and pH 5 solutions. The total root systems grown in the pH 6 solutions were the largest.

Nitrogen absorption.—The absorption rates in terms of milligrams per 100 gm. of green tissue in 6 hours, as listed in table 3, are represented diagrammatically in figure 1. Here, the bars on the left of the vertical zero line represent the average values of nitrogen absorbed as the anion (NO_3-) in milligrams per 100 gm. of green plant tissue during the absorption interval of 6 hours, and

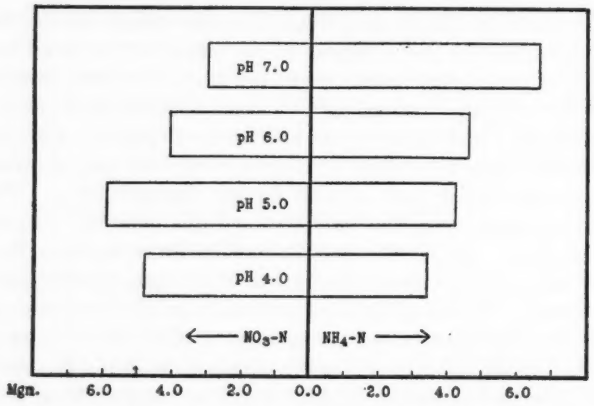

FIG. 1. MILLIGRAMS OF NITROGEN ABSORBED PER 100 GM. GREEN TISSUE IN 6 HOURS BY PLANTS 41 DAYS OLD

those on the right represent the corresponding cation (NH_4^+) absorption values.

Figure 1 brings out clearly the pronounced influence of the pH of the culture solution on the rates of absorption of NH_4-N and NO_3-N when these two forms are present simultaneously in the culture solution in approximately equal proportions.

It is evident that nitrogen in the cation form (NH_4^+) was absorbed much more readily when the pH of the culture solution was relatively high (pH 7) than it was when the pH of the solution was relatively low (pH 4). The rate of absorption of the cation of pH 7 was nearly double that at pH 4. Conversely, nitrogen in the anion form (NO_3^-) was absorbed much more readily at a relatively low pH (pH 4 to pH 5) of the culture solution than it was at the high pH 7. The rate of absorption of the anion at pH 7 was about one-half that at pH 5. The apparent exception to the general trend, indicated by the lower

rate of NO_3-N absorption at pH 4 than at pH 5, may have been due to a slightly unfavorable influence of the pH 4 solution on some phase of the metabolism of the plants, an influence which, in turn, might have affected the rate of absorption of NO_3-N. A suggestion of such an influence was observed in connection with the analytical results, which showed that the soluble organic nitrogen in the roots of the plants grown in the pH 4 solution was noticeably low in comparison with that present in the roots of plants grown in the pH 5, pH 6, and pH 7 solutions.

Furthermore, it is evident from figure 1, that at pH 4 the rate of absorption of NH_4-N was lower than that of NO_3-N, whereas at pH 7 the rate of absorption of NH_4-N was much higher than that of NO_3-N. It should be pointed out here that the data of table 2 in comparison with those of table 3 give evidence of the relationship between the relative rates of absorption of nitrogen as cations and anions and the changes in pH of the culture solutions. It is recognized that data are available for only two of the ions in the culture solution, but it is interesting to note that when the rate of removal of NO_3-N, the anion, from the solution clearly exceeded the rate of removal of NH_4-N, the cation, the pH of the solution increased; whereas, when the rate of removal of the cation distinctly exceeded that of the anion, the pH of the solution decreased. Also when nearly equal absorption rates prevailed, the pH of the solution remained nearly the same during the absorption test interval. Theron (43) has listed several ways in which this relationship could be interpreted.

Results with plants 52 days old

Experimental procedure.—The second absorption test was made 11 days after the first test, with plants grown at the same time and treated in exactly the same manner as were those of the preceding series harvested after the first absorption test had been completed. Solutions at two pH values only, pH 4 and pH 7, were here employed. In addition, several transfers were made 12 days before this absorption test was begun. Triplicate cultures previously grown in the pH 7 solution were transferred to the pH 4 solution and grown in the latter for the remainder of the experimental period. Likewise, triplicate cultures previously grown in the pH 4 solution were transferred to the pH 7 solution and grown in it to the end of the experimental period. The remainder of the cultures of this series grown in both the pH 4 and pH 7 solutions were not transferred, so that a comparison could be made between cultures grown continuously for 25 days in the pH 4 and pH 7 solutions, and cultures grown in these solutions for the first 13 days and then transferred to solutions of opposite reaction for the final 12 days. In every case, the absorption by the plants was tested in a solution of the same pH as that in which they had been grown for the final 12 days.

The plants used for this second absorption test had grown considerably during the 12 days after the transfers were made. Accordingly, 5 days before the second absorption test was begun, the rate of continuous renewal of the

culture solutions had been increased to 2 liters in 14 hours. Even this rate of solution renewal was not great enough to prevent a slightly greater change in pH of the solutions than that caused by the younger plants of the preceding series. This fact is brought out by table 4, in which the values presented were obtained in exactly the same manner as were those given in table 2. It will be observed that the plants 52 days old caused a change in pH of the solutions which was of the same order, although differing slightly in degree, as that caused by the plants 41 days old.

Certain modifications in technique were necessary in testing the absorption rates of the larger plants of this series. Two liters of test solution was used for each culture during the absorption interval instead of the 1 liter used with

TABLE 4

Changes in pH of the culture solutions brought about by plants 52 days old

ORIGINAL pH OF SOLUTION IN RESERVOIRS	pH OF SOLUTIONS FROM OUTLETS OF CULTURE VESSELS BEFORE ABSORPTION TEST	FINAL pH OF SOLUTIONS USED FOR ABSORPTION TEST
4.0	4.0–4.2	4.96–5.5
7.0	5.4–5.8	6.1 –6.3
7.0	5.2–5.75	6.0 –6.15
4.0	4.1–4.2	5.2 –5.6

TABLE 5

Rates of absorption of NH_4-N and NO_3-N by plants 52 days old

pH OF CULTURE SOLUTIONS		AVERAGE GREEN WEIGHT PER PLANT	N ABSORBED PER 100 GM. GREEN TISSUE IN 6 HOURS		
Original	Final 12 days and test		NH_4-N	NO_3-N	Total
		gm.	*mgm.*	*mgm.*	*mgm.*
4.0	4.0	139.0	2.2	7.6	9.8
4.0	7.0	140.9	5.1	5.2	10.3
7.0	7.0	117.8	5.0	8.4	13.4
7.0	4.0	126.3	2.4	5.7	8.1

the preceding series. Of this, 850 cc. was placed in each of the quart jars used as culture vessels in this test. The remainder was dripped through the culture vessel during the absorption interval of 6 hours, so that the rate of renewal was approximately the same as that prevailing before the test. This was about twice the rate of renewal employed with the cultures of the preceding series during the absorption interval.

Each datum in table 5 is the average for triplicate cultures including three plants each. The same units and method of calculation were employed as for the data of table 3. Although the data in table 5 are based on the absorption from twice the amount of solution used per culture for the test of the preceding series, the solution volume per unit of plant tissue was approximately the same in both cases.

Appearance of plants.—Table 5 indicates differences in the average green weights per plant, but the differences probably should not be interpreted as resulting from the pH treatments received during the final 12 days of the experimental period. Instead, they might be traced to the slightly greater size of the plants grown in the pH 4 solution during the early part of the experimental period. However, the plants were all vigorous, and those of each culture in the series had from one to six open blossoms. The roots were healthy and growing, although they were not so white as those of the younger plants of the preceding series. In particular, a light brown tinge was evident on those grown in the pH 7 solution from the beginning of the experimental period.

Nitrogen absorption—The absorption rates listed in table 5 are represented diagrammatically in figure 2, in which the same method of expressing results

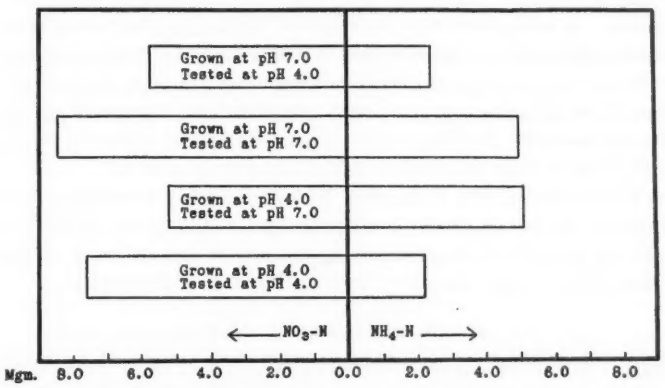
Grown at pH 7.0
Tested at pH 4.0
Grown at pH 7.0
Tested at pH 7.0
Grown at pH 4.0
Tested at pH 7.0
Grown at pH 4.0
Tested at pH 4.0
NO_3-N
NH_4-N
Mgm. 8.0 6.0 4.0 2.0 0.0 2.0 4.0 6.0 8.0

FIG. 2. MILLIGRAMS OF NITROGEN ABSORBED PER 100 GM. GREEN TISSUE IN 6 HOURS BY PLANTS 52 DAYS OLD

Transfers as indicated in the diagram were made 12 days before the absorption tests were begun.

is employed as in figure 1, with the addition of notes indicating the previous history of the plants. The results shown in table 5 and figure 2 will first be examined alone and then compared with those in table 3 and figure 1.

In the first place, the influence of the pH of the solution on the rate of absorption of NH_4-N, the cation, by the plants 52 days old is quite evident. The pH of the culture solution in which the plants were grown during the final 12 days of the experimental period determined the rate of absorption of NH_4-N, as is clearly indicated in figure 2. Those plants grown during the final 12 days and tested in the pH 7 solutions absorbed about twice as much NH_4-N as those grown during the same period and tested in the pH 4 solutions. Thus, it is clear that high rates of absorption of NH_4-N correspond to solutions of high pH values, and low rates of absorption of NH_4-N correspond to solutions of low pH values. This relationship is quite definite.

On the other hand, the average absorption rates of NO_3-N indicated for plants grown continuously in solutions at pH 7 differed only slightly from those indicated for plants grown continuously at pH 4, the advantage being in favor of those grown at pH 7. The absorption rates of NO_3-N were relatively high for these two sets of cultures. The data indicate that when the plants were at this stage of development the pH value of the culture medium, within the limit employed, did not exercise the same dominating influence in determining the rates of NO_3-N absorption as it did in determining the rates of NH_4-N absorption.

The plants which were transferred from solutions at pH 7 to those at pH 4, as well as those which were transferred from solutions at pH 4 to solutions at pH 7, 12 days prior to the time when the absorption tests were made, showed much lower average rates of NO_3-N absorption than did plants grown continuously in solutions having the same pH values as those in which the tests were made. It is apparent that the effect of transferring the plants from solutions of one pH to those of another was to retard the rates of NO_3-N absorption, regardless of whether the transfers were made from solutions of relatively high to those of low pH values, or vice versa. Furthermore, this retarding influence appears to have been effective over a considerable period of time, in the present instance, 12 days from the time when the transfers were made.

The pH of the solution thus seems to have been the dominating factor in determining the rate of absorption of nitrogen as the cation (NH_4^+), but it was not the main factor in determining the rate of absorption of nitrogen as the anion (NO_3^-) when the plants were at this stage of development.

Results with plants 38 days old

Further evidence in support of the foregoing conclusions is presented in table 6 and in figure 3, which include the results obtained with a supplementary series of cultures, in which the plants of one set of cultures were transferred from solutions at pH 7 in which they had been grown continuously to solutions at pH 4 for immediate tests of the absorption rates. Also, another set of cultures was transferred from solutions at pH 4 in which they had been grown continuously to solutions at pH 7 for immediate tests of the absorption rates. The main purpose of the tests was to determine whether change in absorption rates with alteration in the pH values of the culture solutions, as indicated for the plants in the preceding series, is an immediate effect or whether the rate change due to the change in pH of the culture solution is a gradual process of adjustment taking place over a relatively long interval of time. These particular tests were made with plants 38 days old, grown in the same manner as were those of the two series already described, but grown in midsummer, whereas those of the two preceding series were grown during the spring months. The results are quite comparable, although a different unit of plant tissue is used as a basis for expressing the absorption rates, a change made necessary by the lack of data on the green weight of the roots.

As is indicated by the data of table 6, represented diagrammatically in figure 3, the immediate and decisive effect of the pH value of the culture solution on the rates of absorption of NH_4-N is unquestionable. Plants grown in the pH 4 solutions and transferred to and tested in the pH 7 solutions absorbed four times as much NH_4-N during the 6-hour test interval as did those grown and tested in the pH 4 solution. In fact, the average rate of absorption of NH_4-N by the plants so transferred closely approximated that of plants grown and tested in the pH 7 solution. On the other hand, the plants grown in the solution at pH 7 and then transferred to and tested in the pH 4 solution absorbed less than half as much NH_4-N as did those grown and tested in the pH 7 solution. However, the average rate of the absorption of NH_4-N by the plants thus transferred was still considerably greater than that of plants grown and tested in the pH 4 solutions. Here, again, high average rates of absorption of NH_4-N correspond

TABLE 6

Rates of absorption of NH_4-N and NO_3-N by plants 38 days old. Transfers made at the time of the absorption test

pH OF SOLUTION		N ABSORBED PER GM. DRIED TISSUE IN 6 HOURS		
Before test	During test	NH_4-N	NO_3-N	Total
		mgm.	*mgm.*	*mgm.*
4	4	0.25	1.13	1.38
4	7	0.99	0.70	1.69
7	4	0.46	0.81	1.27
7	7	1.09	0.62	1.71

to relatively high pH values of the culture medium, and low rates correspond to low pH values of the medium.

When studying the rates of absorption of NO_3-N by those cultures in which no transfers were made just prior to the time of making the absorption tests, it is found that the opposite relation holds between pH of the medium and average rates of NO_3-N absorption. That is, high average rates of NO_3-N absorption correspond to low pH values of the culture solution and low rates correspond to high pH values, although this relation is not always so clearly defined as is the opposite relation between pH of the culture solution and NH_4-N absorption. With these plants, the disturbing influence of pronounced reaction change of the culture medium upon the NO_3-N absorption rates is very apparent. This is clearly brought out by the fact that the plants grown in solutions at pH 7, then transferred to and tested in solutions at pH 4, showed much lower NO_3-N absorption rates than did the plants grown and tested in solutions at pH 4.

If now, the NH_4-N and NO_3-N absorption rates of the plants transferred from the pH 4 to the pH 7 solutions, as shown in figure 3, are compared with the corresponding rates of the plants transferred from the pH 7 to the pH 4

solutions, it is clear that the great difference between the average NH_4-N absorption rates for these two sets of cultures emphasizes the immediate and determinative effect of the pH of the solution on the rate of absorption of the cation. Although the immediate and determinative influence of pH of the culture solution on the NO_3-N absorption rates is also apparent, the effect is not so pronounced nor so clearly defined as it is in the case of the cation absorption rates.

Influence of age of plants upon absorption

If the data of the older plants (table 5 and figure 2) are considered again in comparison with the data of the younger plants (table 3 and figure 1) the following points of agreement may be observed: (*a*) the average absorption rates of

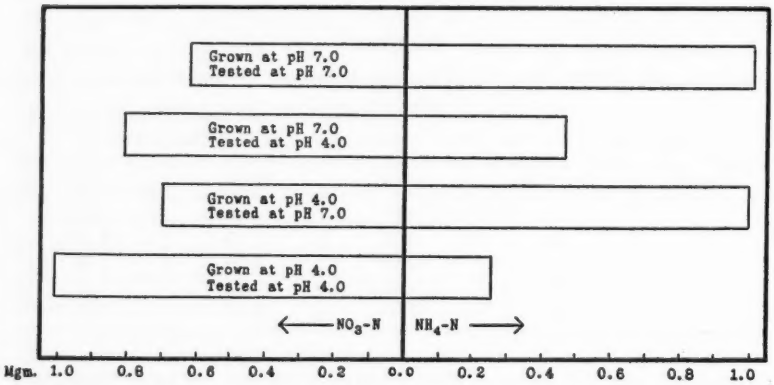
Grown at pH 7.0
Tested at pH 7.0
Grown at pH 7.0
Tested at pH 4.0
Grown at pH 4.0
Tested at pH 7.0
Grown at pH 4.0
Tested at pH 4.0
←NO3-N
NH4-N→
Mgm. 1.0 0.8 0.6 0.4 0.2 0.0 0.2 0.4 0.6 0.8 1.0

FIG. 3. MILLIGRAMS OF NITROGEN ABSORBED PER GRAM DRY PLANT TISSUE IN 6 HOURS BY PLANTS 38 DAYS OLD

Absorption tests were begun immediately after the transfers, as indicated in the diagram, had been made.

NH_4-N were greater at high than at low pH values of the culture solutions, and (*b*), the average rates of absorption of NH_4-N were always lower than were those of NO_3-N from the pH 4 solutions. These points of agreement are due largely to the determinative effect of the pH of the culture solutions on the rates of absorption of NH_4-N.

A further comparison of these data brings out the following points of apparent disagreement: (*a*) The rate of absorption of NO_3-N by the older plants was not greatly influenced by the pH of the solution, which is in direct contrast to the results obtained with the younger plants; and (*b*) the rates of absorption of NO_3-N from the pH 7 solutions by the older plants were always higher than the rates of absorption of NH_4-N from the corresponding solutions, whereas with the younger plants the rates of absorption of NH_4-N from pH 7 solutions always greatly exceeded those of NO_3-N. The cause of these two differences

between the older and younger plants must be sought in the influence of the age of the plants upon the rates of absorption of NH_4-N and NO_3-N.

The results here reported on the effect of the age of the plant on the absorption rates of NH_4-N and NO_3-N are in excellent agreement with those of earlier studies by Jones and Skinner (17); Maftel (27); Prince, Jones, and Shive (35); and Stahl and Shive (41, 42). For example, Stahl and Shive, using a similar technique with buckwheat, found that the rate of absorption of NH_4-N per unit of plant tissue decreased as the plants grew older, whereas the rate of absorption of NO_3-N per unit of plant tissue increased until the flowering stage was reached. Similarly, with the tomato plants here investigated, the data for those grown in both the pH 4 and the pH 7 solutions show that the rates of absorption of NH_4-N decreased as the plants became older and the rates of absorption of NO_3-N increased. A full explanation of these facts is not yet possible.

THEORETICAL CONSIDERATIONS

In these experiments, no attempt was made to study the rates of absorption of any other ions than the NH_4-ion and the NO_3-ion from the culture solution, nor was any study made of the possible excretion of ions or molecules by the plant. The results, therefore, are not complete enough in themselves to furnish the basis for any theory concerning the absorption and assimilation of elements by plants. Nevertheless, the pronounced influence of the pH of the culture medium upon the rates of absorption of NH_4-N and NO_3-N leads to a consideration of the relationships of the results here obtained to theories which have been proposed relative to ion absorption.

In the results presented, the absorption of NH_4-N and of NO_3-N has been referred to as an absorption of ions. Yet it is recognized that the absorption of molecules by plant cells can not be excluded from consideration. Thus Osterhout (29) was led to believe from his work with *Valonia* that either it is principally molecules that penetrate the protoplasm, or that concentration (and perhaps reaction) in the protoplasm plays a more important rôle than diffusion constants. He does not imply that ionic penetration does not occur, but only that it must play a subordinate rôle because of the non-aqueous character of the protoplasmic surfaces.

Brooks (6) states that no non-aqueous solvents have been shown to be more permeable to KOH than to NaOH, as is apparently demanded by Osterhout's molecular hypothesis. Brooks (5) has advanced a hypothesis of ionic exchange. He suggests that the cell is surrounded by a plasma membrane consisting of a mosaic of anion-permeable and cation-permeable areas which are of the nature of charged porous films, that the diameters of the pores are variable according to experimental conditions, and that the penetrabilities of different ions are characteristic functions of their own effective diameters and of the diameters of the pores in the membranes. But he has admitted (6) that

no test permitting experimental discrimination between his hypothesis and that of Osterhout has yet been applied.

Osterhout (29) has criticized Brook's hypothesis for its assumption that anions could not accompany the H ion out of the cell and the subsequent use of the anions to calculate the passage of the Cl ion into the cell. Briggs (2) has given a mathematical treatment of the theoretical case of a membrane having certain areas permeable to anions and certain areas permeable to cations. This calculation casts doubt on the hypothesis of Brooks (5). Briggs (3) has also criticized Osterhout's mathematical formulation of the thermodynamic potential for molecular penetration by pointing out that although the formula would account for the accumulation of K within the cells, no mechanism is suggested for forcing HCl from the outside to the inside of *Valonia* cells against a thermodynamic potential.

Briggs and Petrie (4) and Briggs (2, 3), in turn, have offered several related but different hypotheses based on experiments on the absorption of salts by storage tissue, such as that from potato tubers. This is a special case of absorption, however, and since they did not study the same type of process as that reported in this paper, no comparisons will be attempted.

Butkewitsch (7) studied the passage of salts through collodion membranes and found that if the H-ion concentration of the solution containing the salts was increased, the diffusion of anions through the membrane was increased, or if the OH-ion concentration was increased, the diffusion of cations through the membrane was correspondingly increased. He also conducted other experiments with salts including different combinations of anions and cations, and concluded that molecules diffused through the membrane.

On the other hand, Hager and Stollenwerk (10) quoted an experiment by Michaelis and Fujeta with collodion membranes. Because HCl would not pass through the membrane until a KCl solution was substituted for water on the opposite side of the membrane, they concluded that the H ions could not pass the membranes until K ions were present to provide for an ionic exchange.

Mevius (24, 25) has interpreted his results with maize plants grown in solutions of different pH values as indicating that it is the NH_3 or NH_4OH molecule which penetrates the cell and causes toxicity when the "NH_3-tension" is too great on the outside. He pointed out (25), correctly, that the concentration of undissociated NH_4OH increases with increasing pH of the solution or with increasing concentration of an ammonium salt in a solution. Evidences of the rapid penetration of NH_3 or NH_4OH into tissues have been presented by Harvey (11).

Hager and Stollenwerk (10) supported the view of Mevius (23) regarding the penetration of NH_4OH into cells, and elaborated upon his hypothesis by noting the effect of the hydration of the anion of the ammonium salt upon the penetration of ammonia into the plant. They suggested the term "membrane hydrolysis" a hydrolytic cleavage of the salts which have highly hydrated anions and a subsequent absorption of free ammonia without the anion. However,

in their discussion of the absorption of various forms of ions from solutions, Hager and Stollenwerk did not exclude the possibility of ion-exchange, especially with the H ions and HCO_3 ions of the cell.

Pantanelli (31), after many experiments on the absorption of ions by plants, concluded that normal, living, uninjured protoplasm absorbs individual ions by adsorption, saying that the assumption of a separate absorption of free acids and bases is superfluous, since the natural electric charge of the plasma colloid produces an adsorption potential leading to an ionic separation. He has admitted, however, that it is well known that an entire series of non-dissociated or weakly dissociated organic substances are taken up by the cell in molecular form.

Jenny and Cowan (16) studied the removal by plants of Ca ions adsorbed on purified colloids and concluded that the plant obtained the Ca ions by an excretion of H ions; that is, that an ionic exchange between plant and colloid medium took place.

It is evident that there are supporters of both conceptions, of ionic or molecular absorption of elements by plants, and that there is much to be said in support of both sides of the question.

It is interesting, in this connection, to speculate with reference to how the influence of the pH of the culture solutions here employed on the rates of absorption of NH_4-N and of NO_3-N could be interpreted according to the various theories mentioned. According to Osterhout's (29) theory of molecular absorption, the thermodynamic potential, represented by the product of the activities of the ions (NH_4^+) (OH^-), would increase on the outside of the plant as the OH-ion concentration increased. This would tend to force more NH_4OH into the cell from a solution of high pH than from one of low pH. This is in direct accord with observation in these experiments. Conversely, a similar method of reasoning would lead to the conclusion that the thermodynamic potential (H^+) (NO_3^-) would be greater at a low pH than at a high pH, so that HNO_3 would be absorbed more rapidly from a solution of low pH than from a solution of high pH. This is also in accord with experimental evidence obtained with the younger plants in the present work.

The theory of Mevius (25), that a greater "NH_3-tension" would result from the greater hydrolysis of an ammonium salt at a high pH than would occur at a low pH, and hence, that a higher rate of absorption of NH_3 would take place from a solution of high pH than of low pH, is likewise supported by the experimental data here provided.

Qualitatively, these data appear to be consistent with the theories outlined. Yet it should be pointed out that an increase of only 2 pH units in the culture solution would increase the thermodynamic potential suggested by Osterhout times, or would increase the concentration of un-ionized, NH_4OH emphasized by Mevius nearly 100 times. Yet the rate of absorption of NH_4-N from the pH 7 solutions was never more than four times that from the pH 4 solutions. It would appear, therefore, that the rates of absorption of NH_4-N and NO_3-N

are conditioned by other factors than the thermodynamic potential suggested by Osterhout, or the molecular concentration suggested by Mevius.

It is not possible to correlate in any satisfactory manner the theories of Brooks (5) or of Briggs and Petrie (4) and Briggs (2, 3) with the data here presented.

Attempts have been made to apply the Donnan membrane equilibrium to the absorption of ions by plants. For example, Osterhout (29) has shown that an attempt to extend the Donnan principle to the distribution of electrolytes within and without the cells of *Valonia* is unsuccessful.

Briggs and Petrie (4) also found that the conception of a simple Donnan membrane equilibrium operating between two homogeneous phases is inadequate to explain the phenomena of ionic intake by plants. They extended the scope of the Donnan principle by postulating a greater number of phases in the tissue.

Mattson (21) has applied the laws governing the Donnan equilibrium to the case of a colloidal micelle where an actual membrane does not exist. The success of this application suggests that Donnan equilibria might exist around colloidal micelles in the protoplast. However, the possible existence of more than one equilibrium and the presence of many ions in the solution make it impossible to apply the Donnan equilibrium to the data presented above.

Pantanelli's (31) theory of absorption emphasizes the colloidal properties of the protoplast, and regards absorption as an adsorption process. It appears that any discussion of the absorption or assimilation of elements ought not to neglect the colloidial nature of the protoplast. Heilbrun (12) states that the charge on the outer surface of a cell would be largely determined by ions present in the external medium. Briggs (3) quotes Stiles as suggesting that the relation between the amount of ions absorbed per unit volume of tissue and the final concentration is expressed by the Freundlich isotherm for absorption and that, hence, absorption can not be a simple process of diffusion through a membrane.

In support of these ideas, Robbins (37) found that potato tuber tissue behaves like an ampholyte with an isoelectric point near pH 6.4, basic dyes being absorbed and more strongly retained above pH 6, and acid dyes being absorbed and more strongly retained below pH 6. However, he noted that the absorption of either class of dyes did not stop at the isoelectric point, but continued over to the other side of the possible isoelectric point.

Tiedjens[3] has extended Raber's (36) theory of permeability, which is based on the charge on the cell membrane, to apply to the protoplast as a whole. Considering the protoplast as a whole, Tiedjens pointed out the possible effect of the pH of the culture solution on the dispersion of protein complexes and on the dissociation of soluble ampholytes in the protoplast, and correlated these possi-

[3] Tiedjens, V. A. Factors affecting assimilation of ammonium and nitrate nitrogen, particularly in the tomato (*Lycopersicum esculentum*, Mill.) and in the apple (*Pyrus malus*, L.). Unpublished data.

ble effects with the data he obtained from tomatoes grown in solutions of different pH values. He found that NH_4-N was best assimilated when the pH value of the culture solution was above that of the plant sap, whereas NO_3-N was best assimilated when the pH value of the culture solution was below that of the composite plant sap. He suggested that those ampholytes of the cell which were in a medium above their isoelectric points would combine with cations, such as NH_4^+, whereas those ampholytes in a medium below their isoelectric points would combine with anions, such as NO_3^-. Hence, when the pH of the solution was high, combination with cations was promoted, and when the pH of the solution was low, combination with anions was favored.

This theory, it should be noted, implies that assimilation must take place near the surface of the protoplast which is in contact with the culture medium, unless the cell solution bathing the protein complexes or including the soluble ampholytes within the protoplast is also affected by the pH of the external culture solution. Theron (43) found that the pH of the juices from the tops of plants grown in solutions of different pH values was not affected by the pH of the culture solutions, but that the pH of the expressed sap from the roots was influenced by that of the culture solutions. When the pH range of the external solutions was from 3.5 to 8.5 he found that the maximum range in the juices from the roots was about 1 pH unit. Keysener (19) also found a difference in pH of the root juices of plants grown in culture solutions of different pH values. It is conceivable that the magnitude of difference in pH of the external solutions might be enough to influence the rates of absorption and assimilation of NH_4-N and of NO_3-N as suggested by Tiedjens. The data reported in this paper are in harmony with the theory emphasized by Tiedjens.

Mention should also be made of the work of Niklewski, Krouse, and Lemanczyk (28). They interpreted their results by asserting that the permeability of the plasma membranes depends upon various fluctuations in the degree of dispersion of the plasma, which is influenced by the mineral constituents taken up by the cells. They considered the root colloids to be negatively charged above pH 4 in solutions of $Ca(NO_3)_2$ in order to explain the greater absorption of Ca^+ than of NO_3^- from solutions above pH 4.

Unfortunately, a quantitative comparison of the aforementioned colloidal theories of absorption and assimilation with the data obtained in the experiments here considered can not be made, since the theories are not stated in quantitative form.

It is not at all surprising that no simple and complete picture of the mechanism of the influence of the pH of the culture solution on the rates of absorption and assimilation of NH_4-N and NO_3-N can be presented. Each theory discussed emphasizes only relatively few of the total number of components of the system composed of the plant and its environment, showing how these few components might be affected by a change in the pH of the culture solution. But the plant and its environment make up a complex system with many components which may possibly be affected by a change in any one of them.

Thus changing the pH of the culture solution does affect the ionic and dissociation equilibria within that solution and may conceivably affect such things as the electrostatic charges on the surface of the protoplasm and on the interior of the protoplast, the degree of dispersion of the colloidal complexes of the protoplast, the dissociation of ampholytes within the protoplasm, the pH of the composite sap of the roots as a whole or of specific tissues therein, the buffer systems of the roots, and the CO_2 or HCO_3^- equilibrium between the plant and the solution. Furthermore, a change in any one of these components may influence one or more of the other components in a secondary way. Indeed, on account of the complexity of the system, any attempt to interpret all of its activities based only on the behavior of a few of the active components of the whole system must of necessity be incomplete.

SUMMARY

Tomato plants were grown in continuously renewed and continuously aerated, complete culture solutions including approximately equal proportions of NH_4-N and NO_3-N, the solutions being adjusted to pH 4.0, 5.0, 6.0, and 7.0. The rates of absorption of NH_4-N and of NO_3-N from the solutions by the plants were determined at two different ages. In addition, the plants were harvested at the time of each absorption test and analyses of the tissue for certain nitrogenous fractions were made. The following results were obtained:

Absorption by plants 41 days old

The rates of absorption of NH_4-N were higher from solutions of high pH than from solutions of low pH, the rates of absorption at pH 7 being nearly twice those at pH 4.

The rates of absorption of NO_3-N were higher from solutions of low pH than from solutions of high pH, the rates of absorption at pH 7 being about one-half those at pH 5, where the maximum rates occurred.

At pH 4 the NH_4-N absorption rates were lower than the NO_3-N rates. At pH 7 the NH_4-N absorption rates were higher than the NO_3-N rates.

The maximum rates of NH_4-N absorption were higher than the maximum rates of NO_3-N absorption in the younger plants.

Absorption by plants 52 days old

In agreement with the results obtained with the plants 41 days old, the rates of absorption of NH_4-N were higher at pH 7 than at pH 4.

The rates of absorption of NH_4-N were much lower than those of NO_3-N from the pH 4 solutions. These similarities were due to the determinative effect of the pH of the solutions on the rates of nitrogen absorption.

In contrast with the results obtained with the younger plants, the rates of absorption of NO_3-N in the older plants were not so greatly influenced by the pH of the solution as they were in the younger plants.

Within the range of pH values employed, the rates of absorption of NO_3-N

were higher than those of NH_4-N absorption from solutions of corresponding pH. This emphasizes the influence of the age of the plants upon the relative rates of absorption of NH_4-N and NO_3-N.

Within the period investigated, the rates of absorption of NH_4-N per unit of plant tissue decreased, and the rates of absorption of NO_3-N increased as the plants became older.

Effect of reaction change

The effect of transferring plants from solutions of one pH to those of another pH, 12 days before the absorption test, was to retard the rates of absorption of NO_3-N, regardless of whether the transfers were made from solutions of relatively high to those of low pH values, or vice versa.

Plants transferred from pH 7 solutions to pH 4 solutions or vice versa for immediate absorption tests, showed that the effect of the pH on the rates of absorption of NH_4-N was immediate and decisive.

Although reaction change exerted an immediate and marked disturbing influence upon the rates of NO_3-N absorption, the effect was not so pronounced or so clearly defined as it was upon the rates of NH_4-N absorption.

Outstanding theories of absorption and permeability are discussed in relation to their bearing upon the results obtained in these investigations.

REFERENCES

(1) ALLISON, R. V. 1922 The relation of aeration to the development of the soybean plant in artificial culture. *N. J. Agr. Exp. Sta. Ann. Rept.*, 1921, 338–344.

(2) BRIGGS, G. E. 1930 The accumulation of electrolytes in plant cells: a suggested mechanism. *Proc. Roy. Soc. (London) Ser. B* 107: 248–268.

(3) BRIGGS, G. E. 1932 The absorption of salts by plant tissues, considered as ionic interchange. *Ann. Bot.* 46: 301–322.

(4) BRIGGS, G. E., AND PETRIE, A. H. K. 1928 On the application of the Donnan equilibrium to the ionic relations of plant tissues. *Biochem. Jour.* 22: 1071–1082.

(5) BROOKS, S. C. 1929 The accumulation of ions in living cells—a non-equilibrium condition. *Protoplasma* 8: 389–412.

(6) BROOKS, S. C. 1931 The accumulation of strong electrolytes in living cells. *Science* 73: 589–590.

(7) BUTKEWITSCH, W. W. 1929 Zur Frage über den Mechanismus der Nährstoffaufnahme durch die Pflanze. *Landw. Jahrb.* 69: 521–541.

(8) CLARK, H. E., AND SHIVE, J. W. 1932 Influence of continuous aeration upon the growth of tomato plants in solution cultures. *Soil Sci.* 34: 37–41.

(9) CLARK, W. M. 1928 The determination of Hydrogen Ions, ed. 3. Baltimore, Md.

(10) HAGER, G., AND STOLLENWERK, W. 1920 Ein Beitrag zur Erkenntnis der Nährstoffaufnahme durch die Pflanzen. *Ztschr. Pflanzenernähr., Düng., u. Bodenk.* (A) 17: 129–165.

(11) HARVEY, E. N. 1913 A criticism of the indicator method of determining cell permeability for alkalis. *Amer. Jour. Physiol.* 31: 335–342.

(12) HEILBRUN, L. V. 1928 The Colloid Chemistry of Protoplasm. Protoplasmamonographien, vol. 1. Berlin.

(13) HOAGLAND, D. R. 1919 Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant. *Jour. Agr. Res.* 18: 73–117.

(14) HUTCHINSON, H. B., AND MILLER, N. H. J. 1909 Direct assimilation of ammonium salts by plants. *Jour. Agr. Sci.* 3: 179–193.

(15) HUTCHINSON, H. B., AND MILLER, N. H. J. 1911 The direct assimilation of inorganic and organic forms of nitrogen by higher plants. *Centbl. Bakt.* (II) 30: 513–540.

(16) JENNY, H., AND COWAN, R. W. 1933 The utilization of absorbed ions by plants. *Science* 77: 394–396.

(17) JONES, C. D., AND SKINNER, C. E. 1927 Absorption of nitrogen from culture solutions by plants. *N. J. Agr. Exp. Sta. Ann. Rept.* 1926, 360–365.

(18) JONES, L. H., AND SHIVE, J. W. 1921 Effect of ammonium sulfate upon plants in nutrient solutions supplied with ferric phosphate and ferrous sulfate as sources of iron. *Jour. Agr. Res.* 21: 701–728.

(19) KEYSENER, E. 1931 Der Einfluss der Wasserstoffionenkonzentration in der Nährlosung auf die Reaktion in der Pflanze. *Planta* 12: 575, 587.

(20) LOO, T. L. 1931 Studies on the absorption of ammonia and nitrate by the roots of *Zea mays* seedlings, in relation to the concentration and the actual acidity of culture solution. *Jour. Fac. Agr., Hokkaido Imp. Univ.* 30: 1–118.

(21) MATTSON, S. 1929 The laws of soil colloidal behavior: I. *Soil Sci.* 28: 179–220.

(22) MAZE, P. 1898 L'assimilation de l'azote nitrique et de l'azote ammoniacal par les vegetaux superieurs. *Compt. Rend. Acad. Sci.* (*Paris*) 127: 1031–1033.

(23) MAZE, P. 1900 Recherches sur l'influence de l'azote nitrique et de l'azote ammoniacal sur le developpment du mais. *Ann. Inst., Pasteur* 14: 26–45.

(24) MEVIUS, W. 1927 Bedeutung der Reaktion für die Wirkung der Ammoniumsalze auf das Wachstum von Zea Mays. *Ztschr. Pflanzenernähr., Düng., u. Bodenk.* (A) 10: 208–218.

(25) MEVIUS, W. 1928 Die Wirkung der Ammoniumsalze in ihrer Abhängigkeit von der Wasserstoffionenkonzentration. *Planta* 6: 379–455.

(26) MEVIUS, W., AND ENGEL, H. 1929 Die Wirkung der Ammoniumsalze in ihrer Abhängigkeit von der Wasserstoffionenkonzentration. II. *Planta* 9: 1–83.

(27) NAFTEL, J. A. 1931 The absorption of NH_4- and NO_3-N by various plants at different stages of growth. *Jour. Amer. Soc. Agron.* 23: 142–158.

(28) NIKLEWSKI, B., KROUSE, A., AND LEMANCZYK, K. 1928 Zur Kenntnis der Aufnahmemechanik der Mineralbestandteile durch Wurzeln der Pflanze. *Jahrb. Wiss. Bot.* 69: 101–118.

(29) OSTERHOUT, W. J. V. 1930 The accumulation of electrolytes: II. Suggestions as to the nature of accumulation in Valonia. *Jour. Gen. Physiol.* 14: 285–300.

(30) PANTANELLI, E. 1915 Über Ionenaufnahme. *Jahrb. Wiss. Bot.* 56: 689–733.

(31) PANTANELLI, E. 1929 Über Ionenaufnahme. *Sammelreferat, Protoplasma* 7: 129–137.

(32) PIRSCHLE, K., AND MENGDEHL, H. 1931 Ionenaufnahme aus Salzlösungen durch die höhere Pflanze. *Jahrb. Wiss. Bot.* 74: 297–363.

(33) PRIANISCHNIKOW, D. 1926 Ammoniak, Nitrate, und Nitrite als Stickstoffquellen für höhere Pflanzen. *Ergeb. Biol.* 1: 407–446.

(34) PRIANISCHNIKOW, D. N. 1929 Zur Frage nach der Ammoniakernährung von höheren Pflanzen. *Biochem. Ztschr.* 207: 341–349.

(35) PRINCE, A. L., JONES, L. H., AND SHIVE, J. W. 1923. Notes on differential ion absorption by plants in relation to reaction changes in nutrient solutions. *N. J. Agr. Exp. Sta. Ann. Rept.* 1922: 378–383.

(36) RABER, O. 1923 Permeability of the cell to electrolytes. *Bot. Gaz.* 75: 298–308.

(37) ROBBINS, W. J. 1926 The isoelectric point for plant tissue and its importance in absorption and toxicity. *Univ. Missouri Studies; Quart. Res.* 1: 3–60.

(38) SABININ, D. A., AND KOLOTOVA, S. S. 1927 The character of a passage of ash substances into a plant: 1. The reaction of medium as a factor of mineral nutrition of plants. *People's Comm. of Agr., Agr. Exp. Sta. of Per. Results of Investigations in 1926. Dept. of Agr. Chem., No. 1*, p. 91–107.

(39) SESSIONS, A. C., AND SHIVE, J. W. 1928 A method for the determination of inorganic nitrogen in plant extracts. *Plant Physiol.* 3: 499–511.

(40) SHIVE, J. W., AND STAHL, A. L. 1921 Constant rates of continuous solution renewal for plants in water cultures. *Bot. Gaz.* 84: 317–323.

(41) STAHL, A. L., AND SHIVE, J. W. 1933 Studies on nitrogen absorption from culture solutions: I. Oats. *Soil Sci.* 35: 375–399.

(42) STAHL, A. L., AND SHIVE, J. W. 1933 Further studies on the absorption of nitrogen from culture solutions: II. Buckwheat. *Soil Sci.* 35: 469–483.

(43) THERON, J. J. 1924 Influence of reaction on interrelations between the plant and its culture medium. *Univ. Calif. Pubs., Agr. Sci.* 4: 413–444.

(44) TIEDJENS, V. A., AND ROBBINS, W. R. 1931 The use of ammonia and nitrate nitrogen by certain crop plants. N. J. Agr. Exp. Sta. Bul. 526.

(45) TOTTINGHAM, W. E. 1914 A quantitative chemical and physiological study of nutrient solutions for plant cultures. *Physiol. Res.* 1: 133–245.

A STUDY OF RHIZOBIUM SPECIES IN RELATION TO NODULE FORMATION ON THE ROOTS OF FLORIDA LEGUMES: II[1]

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Received for publication June 26, 1933

NITROGEN-FIXATION STUDIES

The ability of the nodule bacteria of Leguminosae to fix nitrogen is a property of much concern from a practical point of view. It has been studied by several investigators in connection with cultures representing some of the more important cross-inoculation groups. Stevens (17), working with Rhizobia from alfalfa and sweet clover, found certain "sub-strains" to be much more efficient than others in this respect. The power of nodule production was about the same, since the low nitrogen-fixers formed as many nodules per plant as did the ones possessing power of greater nitrogen assimilation. Similar results have been reported by Wright (23) for different cultures of soybean bacteria; by Sears and Clark (14) and by Hansen and Tanner (6) with navy and valentine beans; and by Whiting, Fred, and Helz (22) with cultures isolated from the field pea. In the latter case, inoculation with the more efficient nitrogen fixers raised the percentage of nitrogen, increased the yield, and raised the proportion of fancy table peas (sizes 2 and 3) to low grade peas (sizes 4 and 5).

In view of the aforementioned reports, it seemed desirable to conduct nitrogen-fixing experiments with the cowpea and soybean with certain cultures of their nodule-producing bacteria.

The tests were made with sand cultures in 1-gallon pots. The procedure and technique employed were the same as those described in section I for cross-inoculation work.[3] The experiment for each culture was run in replicates of three pots with three plants each. The plant nutrient administered was Bryan's modification of Crone's nitrogen-free solution, the formula for which has been given elsewhere. The experiments were observed daily, the moisture content corrected to approximately 60 per cent, saturation twice a week, and the nutrient solution given once a week. The duration of the tests was 11 weeks, or up to the fourth or fifth day after the first blooms appeared.

Figure 1 of plate 1 shows a representative one of each replicate of the three pots from seven of the nine tests at this stage of development. In examining

[1] A portion of a thesis submitted in partial fulfilment of the requirements for the degree of doctor of philosophy, August 1932.

[2] The serological work was done in coöperation with Miss Hellen Jeffery (Kentucky Board of Health).

[3] *Soil Sci.* 37 (2), February 1934.

the photograph, it should be borne in mind that all the conditions were constant except that of inoculation, which varied. The plants are arranged in order of

TABLE 1

Efficiency of cowpea and soybean bacteria on cowpea plants

CULTURE USED	REPLICATE NO.	OVEN-DRY WEIGHT OF 3 PLANTS	NITROGEN	NITROGEN IN PLANTS	GAIN IN NITROGEN*
		gm.	*per cent*	*mgm.*	*mgm.*
Controls	1	1.86	1.260	23.4	.....
	2	1.66	1.350	22.4	.....
	3	2.22	1.370	30.4	.....
Soybean 6	1	5.10	3.480	177.5	152.1
	2	6.19	2.650	164.0	138.8
	3	5.48	3.105	170.1	144.7
Cowpea 11	1	6.49	3.125	202.8	177.4
	2	7.63	3.390	258.6	233.2
	3	6.89	3.685	253.9	228.5
Soybean 915	1	6.87	3.075	211.3	185.9
	2	7.86	3.480	273.5	248.1
	3	8.29	2.975	246.6	221.2
Soybean 1	1	6.80	3.970	269.9	244.5
	2	6.71	3.035	203.6	178.2
	3	7.22	3.470	250.5	225.1
Soybean 13	1	8.35	3.835	320.2	294.8
	2	7.99	3.980	318.0	292.6
	3	8.00	3.980	318.4	293.0
Cowpea 10	1	9.15	4.580	419.1	393.7
	2	8.22	4.455	366.2	340.8
	3	8.42	4.370	367.9	342.5
Cowpea 2	1	7.35	4.940	363.0	337.6
	2	8.33	4.615	384.4	359.0
	3	8.93	4.010	358.0	332.6
Cowpea 28	1	8.21	3.675	301.7	276.3
	2	8.90	3.740	332.8	307.4
	3	8.60	3.790	325.9	300.5

* Difference between the average amount of nitrogen in the three control plants (25.4 mgm.) and the amount of nitrogen found in the inoculated plant.

a graduation from the poorest to the best with regard to efficiency of the cultures as nitrogen fixers. The plants in pot 1 (the control) attained most of

their growth during the first 6 weeks. They were yellow and sickly in appearance. Although the photograph does nor show it very well, there was a gradation in intensity of color from left to right, the color range reaching a dark green in pots 6 and 7. The results indicate clearly a variation in the nitrogen-fixing power of the Rhizobia from the nodules of cowpeas and soybeans when working in symbiosis with the cowpea plant.

After the photographs were taken, the plants were harvested; the tops being cut at the surface of the sand. The roots were then washed free of sand, and the nodules per plant counted. The number of nodules ranged from 63 to 105 per plant, without respect to cultures. After being dried over night at 100°C. or slightly above, the plants were weighed, and their nitrogen content was determined by the "Gunning Method." The data from these determinations are given in table 1 and confirm the idea conveyed by the photograph.

Nitrogen-fixing tests similar to these described were conducted with some of the aforementioned cultures and the soybean host plant. Cowpea cultures 2 and 10 had to be omitted, however, since they do not produce nodules on the roots of the soybean plant (just one of the unexplained but common inconsistencies exhibited by the legume nodule bacteria of the cowpea cross-inoculation group). No analyses were run on the soybeans, but every indication is that the results would have been similar to those obtained with cowpeas.

Figure 2 of plate 1 shows these soybeans plants arranged with an uninoculated control at the left, the poorest appearing inoculated plants next in order and on through with the better ones of the group at the right.

The experiment with the cowpeas described in the foregoing was repeated in the greenhouse with a sort of supplementary field test. The greenhouse tests were conducted in duplicate with only five cultures. The field supplement was not conducted entirely in keeping with the custom for such tests. In this case, the extra plants at the time for thinning (6 weeks) in the greenhouse test, were removed from the pots with sterilized forceps and spatulas to small plats prepared in the field. The usual procedure for field tests is to plant the seeds in soil.

All of the inoculated plants showed an abundance of nodules, without respect to cultures used, at the time of transplanting. Two of the cultures used had begun to show signs of superiority (greater plant development) at this time. These continued to develop while the other inoculated plants, as well as the controls, showed a considerable lag period. After approximately 3 weeks, however, these, including the uninoculated controls, darkened in color and developed rather rapidly, as a result, no doubt of inoculation in the soil, until the end of the twelfth week, when all of them were photographed and harvested.

The greenhouse portion of the foregoing experiment was not entirely satisfactory. During the last 3 weeks of the test, the temperature under glass was too high for the best development of the cowpea plants. In spite of this retarded growth, however, the plants show relative proportions and are included in the photograph taken of the field plats (pl. 1, fig. 3).

The uninoculated controls appear at the right of the photograph and are numbered 1. They are not very clear, because of poor light. In each case one of the duplicate pots from which the field plants were taken is elevated behind its respective field plat. The photograph with its legends gives the details concerning cultures used.

Figure 1 of plate 2 showing small plat 5, plants inoculated with cowpea culture no. 31, gives a fair idea of good inoculation under field conditions. The soil adjacent to the plat was removed to a depth of 3½ feet, then the roots were exposed by washing gently with water from a hose.

The foregoing data indicate that only certified nitrogen-fixing Rhizobia should be used in commercial culture work.

SEROLOGICAL PROPERTIES OF RHIZOBIA

In 1912, Zipfel (24) found that the antisera for some cultures of the legume nodule bacteria would not agglutinate certain others. Later (in 1914) Klimmer and Kruger (7), by agglutination and complement-fixation methods, pointed out a correlation between serological groups and Simon's (15) cross-inoculation group. The results of these investigations were confirmed in 1921 by Vogel and Zipfel (19). To this point, data concerning serological and cross-inoculation relationships seemed to be consistent and to coincide with each other.

However, Stevens (16) in 1923, working with 55 cultures representing 7 species of legume bacteria, found that Rhizobia belonging to any cross-inoculation group could be further separated into two or more sub-groups by the agglutination reaction. This investigator (17), in 1925, found differences also in the ability of the subgroups to fix nitrogen in symbiosis with their interchangeable host plants.

Bialosuknia and Klott (3) reported also, in 1923, that the same kind of plant from different sources had nodule bacteria with different agglutination properties. These investigators reported two cultures from the same nodule that differed in this respect.

Dunham and Baldwin (4) isolated two serologically different cultures of Rhizobia from the same leguminous plant, but failed to find more than one type of organism in the same nodule.

Wright (23), Walker (21), Hansen and Tanner (6), and others working along similar lines have confirmed these serological variations.

In considering the references given, it seems that the complement-fixation test was employed in the main for determining the serological relationship of legume nodule bacteria. Later both the complement-fixation and agglutination reactions were used. It seems, however, that in later years workers in this field have resorted more to the latter reaction for serological classification of these bacteria. The difficulty encountered in manipulating the technique of the complement-fixation test undoubtedly accounts for the unpopularity of this reaction. It was decided, however, to use both the tests in connection

with Rhizobia representing the different cross-inoculation groups under consideration here.

The technique followed in carrying out the complement-fixation studies is that described by Kolmer (8) for diagnosing bacterial diseases. In this method, the standardized antigen and complement are mixed with the immune serum in saline and placed in the ice box over night. The hemolytic system is

TABLE 2

Results of complement-fixation tests with five antisera with antigens of various nodule bacteria

ANTIGENS	ANTISERA				
	Bean 80	Clover 5	Cowpea 600	Lespedeza 6	Soybean 504
Alfalfa 2	−	−	0	−	−
Amorpha	−	−	0	−	−
Amphicarpa	+	++	0	++	−
Bean 17	++−	++	0	++	−
Bean 80	++++	−	0	−	−
Bean 405	++++	++	0	−	−
Black locust	−	−	0	−	−
Clover 4	−	++	−	−	−
Clover 5	Ac.	++++	±	−	−
Clover 45	−	±	−	−	−
Clover 200	−	−	−	−	−
Cowpea 600	−	−	++++	−	+
Crotalaria 81	±	−	++	−	−
Dalea	−	−	−	−	+
Lespedeza 6	−	±	+	++++	+
Lotus	++	−	±	−	−
Lupine B	−	−	−	−	+++
Lupine 16	−	−	−	−	+++
Lupine 801	−	−	−	−	−
Lupine 1	−	−	±	−	−
Pea 1	−	−	−	−	−
Sanfoin	−	−	−	−	−
Soybean 5	−	−	−	−	−
Soybean 68	−	−	±	−	+++
Soybean 504	−	−	−	−	++++
Vetch (kidney)	−	−	−	−	−
Vetch (wild)	−	−	−	−	−

Ac. = anticomplementary reaction of antiserum.
0 = no test, or unsatisfactory results.

then added, and the mixture incubated 1 hour at 37°C., at the end of which time the results are recorded.

The antisera used were from rabbits which had been immunized by injecting four doses of approximately 200,000,000 cells of nodule bacteria intraperitoneally at intervals of 6 days, and one half-size dose intravenously 5 days after the fourth intraperitoneal injection. The bacterial suspensions were prepared

by washing off several 5-day-old slant cultures and standardizing by the turbidity method of Gates (5). As the various cultures of Rhizobia differ greatly in opacity, this method was not very satisfactory. Seven days after the last injection the rabbits were bled from the heart. The blood was allowed to coagulate at room temperature. The firm clot was then released from the walls of the test tube with a small sterilized glass rod and the serum separated

TABLE 3

Agglutination reaction of six antisera with various cultures of nodule bacteria

ANTIGEN	ANTISERA					
	Bean 80	Clover 5	Cowpea 600	Lespedeza 6	Soybean 5	Soybean 504
Amorpha	–	–	–	–	–	–
Amphicarpa	–	–	–	–	–	–
Bean 80	1:2560	–	–	–	–	–
Bean 17	1:20	–	–	–	–	–
Bean 405	1:2560	–	–	–	–	–
Beggar-weed 400	–	–	1:2560	1:2560	–	–
Beggar-weed 402	–	–	1:2560	1:2560	–	–
Black locust	1:40	–	–	–	–	–
Clover 4	–	1:2560	–	–	–	–
Clover 5	–	1:2560	–	–	–	–
Clover 45	–	1:80	–	–	–	–
Cowpea 2	–	–	1:2560	1:2560	–	–
Cowpea 10	–	–	1:2560	1:2560	–	–
Cowpea 600	–	–	1:2560	1:2560	–	–
Crotalaria 81	–	–	–	–	–	–
Crotalaria 408	–	–	1:40	–	–	–
Crotalaria 409	–	–	–	–	–	–
Dalea	1:40	–	–	–	–	–
Kidney vetch	–	–	–	–	–	–
Lespedeza 6	–	–	1:1200	1:2560	–	–
Lupinus vilosus 1	–	–	–	–	–	–
Lupine 3	–	–	–	–	–	–
Sanfoin	–	–	–	–	–	–
Soybean 5	–	–	–	–	1:2560	–
Soybean 68	–	–	1:80	–	1:20	1:2560
Soybean 504	–	–	–	–	1:40	1:2560
Wistaria 421	–	–	–	–	–	–

by centrifugal force. The resulting serum was preserved with 0.2 to 0.3 per cent of tricresol. At this point the serum was stored in the ice box until needed.

The results obtained by the application of the two tests mentioned, shown in tables 2 and 3, compare favorably. It will be noted, however, that in certain cases where cultures representing different cross-inoculation groups are

compared, the cross-agglutination test was negative, whereas a ±, +, ++, or even a +++ was obtained in the complement-fixation test. Cowpea 600 gave ++ with the antiserum of clover 5, and lupines 3 and 16 each gave a +++ complement-fixation, with soybean culture 504, but showed negative agglutination with the same antisera. In most other cases, however, the data in one table check with those in the other. Bean culture 17, for example, and bean culture 80, both of the same cross-inoculation group, show good comparative results. The former culture gave good agglutination with a 1:20 dilution of the antiserum of the latter. The corresponding complement-fixation test showed a ++ result. It may be noted that a few extra cultures were added to the list of antigens for agglutination. These tests are of significance in work of this nature, since they aid in the separating of nodule-bacteria of low nitrogen-fixing ability in a given cross-inoculation group from the more effective nitrogen-fixers of the same group.

PROTEIN KINSHIP OF CROTALARIA SPECIES

Baldwin, Fred, and Hastings (1) have shown a fair correlation between protein-kinship of legumes and cross-inoculation groups. Since the Crotalaria species investigated in this work are of special interest and seem to interchange nodule bacteria among themselves and with the cowpea, their protein relationship is of interest. Seeds from only 15 of the 22 species investigated were available, however, so this survey could not include the entire list. Alsike clover and the field pea, each belonging to separate cross-inoculation groups and different from the cowpea group, were tested by way of comparison with Crotalaria species.

In the carrying out of these tests, healthy rabbits were immunized to saline extracts of the ground seeds of alsike clover (*Trifolium hybridum*), cowpea (*Vigna sinensis*), *Crotalaria mundyi*, and the field pea (*Pisum sativum*). The seeds were treated with 70 per cent alcohol for 5 minutes, washed, and ground in a sterile mortar. This macerated material was mixed with approximately 4 volumes of saline and allowed to stand over night in the refrigerator. The supernatant fluid was decanted into sterile test tubes and preserved with tricersol.

The rabbits were given four intraperitoneal injections of 3 cc. of the seed infusion at intervals of 5 days. Seven days after the last injection, they were bled and the antisera collected as described for the complement-fixation tests. The antigens (seed infusions) for carrying out the precipitin tests were filtered and diluted to a point where very little turbidity remained.

The precipitin tests were carried out by the contact method in capillary tubes, the antisera being drawn into the tube, the constricted portion sealed, and the antigen slowly added to form a stratum on the serum. The tests were then incubated 30 to 40 minutes at 37°C. and read. A white ring formed at the point of junction of the two liquids, indicating a positive reaction.

The results of these precipitin tests are recorded in table 4. The density of the precipitate formed by the antiserum with its specific antigen in each case was taken as a standard and indicated by the "four plus" (++++) sign. The other results were given one, two, three, or four plus signs according to the amount of precipitate formed as compared to the standard. The negative sign was used to indicate no precipitate.

These data would indicate in general a close degree of protein kinship among the Crotalaria species tested and, in most cases, with the cowpea. In the case of *Crotalaria lanceolata, polysperma, spectabilis,* and *verrucosa,* the precipitate with the cowpea antiserum was slight or none. These tests were repeated with

TABLE 4

Results of the precipitin test for antisera and 15 species of Crotalaria

ANTIGENS	ANTISERA			
	C. mundyi	Cowpea	Field pea	Alsike clover
C. alata	+++	++	−	+−
C. anagyroides	++++	++	−	−
C. Axillaria	+++	++	−	−
C. goreensis	++	+++	−	−
C. incana	++++	+++	−	−
C. lanceolata	++++	+−	−	−
C. mundyi	++++	+++	±	−
C. mysorensis	++	+++	−	−
C. polysperma	++++	±	−	−
C. retusa	+++	++	−	−
C. spectabilis	++++	−	−	−
C. striata	+++	+	−	−
C. valentonii	+++	+++	−	−
C. vallicola	++++	+++	−	−
C. verrucosa	++++	−	−	−
Alsike clover	+	±	−	++++
Cowpea	−	++++	−	−
Field pea	−	−	++++	−

the same result, and are taken to indicate that only slight protein relationship exists between these Crotalaria species and the cowpea. The reactions of field pea and alsike clover antisera with nonspecific antigens appear to signify that little or no kinship exists between these two plants and the Crotalaria tested.

HYPOTHESIS OF GROUP INOCULATION

The phenomenon of cross-inoculation or group inoculation and the mode of infection have been known (11) and generally accepted for a number of years. The underlying reason for nodule formation by a given culture of Rhizobia on

the roots of a given leguminous plant, or a group of such plants, and for failure of this same culture to accomplish this, in case of other closely related plants, is not understood.

Agricultural bacteriologists generally seem to consider entrance of Rhizobia into the root a true infection as in a case of a parasite in disease. However, in the light of present-day knowledge of plant physiology and plant "makeshifts" in nitrogen utilization, it seems entirely possible that the leguminous plant possesses an enzyme which enables it to use the particular form of organic nitrogen contained in the respective symbiont. The fact that plants have some power of selecting is borne out by the works of Perkins (10) and Voorhees (20) with varieties of the soybean.

The indications that poor inoculation is obtained in the presence of sufficient nitrate nitrogen (for plant development), and that fluctuation of the calcium nitrogen ratio (12) may influence nodule formation, cause the writer to believe that if conditions are such as to enable the plant to use more nitrogen than is available in the soil, the plant becomes infected and uses the bacterial nitrogen. In addition to the aforementioned, certain plants, such as rice, prefer ammonia to nitrate nitrogen (9). Certain other green plants, Venus' fly trap., (*Dionaea muscipula*), sundew (*Drosera intermedia*), and others, are able to entrap insects and utilize the nitrogen. These odd nitrogen "makeshifts" are recognized and accepted by physiologists, and it seems reasonable to assume that the leguminous plant and its respective bacterial symbionts present another striking example of the same thing.

If the hypothesis mentioned should be correct, a question as to whether the plant uses any cycle-stage of the bacteria themselves or their excretions arises. Beijerinck, as well as Bewley and Hutchinson (2) and Thornton (18), describes at least three stages in the life cycle of several species of Rhizobium. Prazmowski (11) and others describe a sheath which surrounds the bacterium soon after the infection takes place. Since the coccoid stage (2) becomes motile and swims about (in cultures at any rate) without a sheath for a time, it may be that these unprotected forms are used by the plant. Figure 2 of plate 2 shows the stages in the life cycle of one of the cowpea nodule bacteria. Since the literature gives full discussions of older theories attempting to explain group-inoculation it need not be reviewed here.

SUMMARY AND CONCLUSIONS

Certain strains of nodule bacteria belonging to the cowpea cross-inoculation group show greater power of nitrogen-fixation than other strains in the group.

The complement-fixation test and the agglutination reaction were compared as methods for separating or classifying strains of nodule bacteria. They appear to be about equal in reliability, but because of its simplicity the agglutination reaction is to be preferred.

A close protein kinship has been shown to exist among the 15 Crotalaria species tested. Most of the species also happen to be related to the cowpea plant. The indication is that none of them is closely related to alsike clover or to the field pea.

REFERENCES

(1) BALDWIN, I. L., FRED, E. B., AND HASTINGS, E. G. 1927 Grouping of legumes according to biological reactions of their seed proteins. *Bot. Gaz.* 83: 217–243.

(2) BEWLEY, W. F., AND HUTCHINSON, N. B. 1920 On the changes through which the nodule organism (Ps. Radicicola) passes under cultural conditions. *Jour. Agr. Sci.* 10: 144–161.

(3) BIALOSUKNIA, W., AND KLOTT, C. 1923 Untersuchungen über Bacterium radicicola. *Rocz. Nauk Rolnicz.* 9: 288–335. (Orig. not read: Abs. *Ber. Gesam. Physiol. u. Exp. Pharmakol.* 29: 14 (1924).)

(4) DUNHAM, D. H., AND BALDWIN, I. L. 1931 Double infection of leguminous plants with good and poor strains of Rhizoba. *Soil Sci.* 32: 235–249.

(5) GATES, F. L. 1920 A method of standardizing bacterial suspensions. *Jour. Exp. Med.* 31: 105.

(6) HANSEN, R., AND TANNER, F. W. 1931 The nodule bacteria of the Leguminosae with special reference to the mechanism of inoculation. Centbl. Bakt. (II) 85: 130–152.

(7) KLIMMER, M., AND KRUGER, R. 1914 Sind die beiden verschieden Leguminosen gefunden Knollchenbakterien artverschiede? *Centbl. Bakt.* (II) 40: 256.

(8) KOLMER, J. A., AND BOERNER, F. 1931 Approved Laboratory Technique, ed. 1, p. 468–472. Appleton Co., New York.

(9) MILLER, E. C. 1931 Nitrogen Metabolism of Green Plants. Plant Physiology, ed. 1. McGraw-Hill Co., New York.

(10) PERKINS, A. T. 1925 Regarding the possible adaptation of soybean radicicola to a specific host variety. *Jour. Agr. Res.* 30: 243–244.

(11) PRAZMOWSKI, A. 1890 Die wurzelknollchen der Erbse. *Landw. Vers. Sta.* 37: 160–238.

(12) SCANLAN, R. W. 1928 Calcium as a factor in soybean inoculation. *Soil Sci.* 25: 313.

(13) SEARS, O. H., AND CARROLL, W. R. 1927 Cross-inoculation with cowpea and soybean nodule bacteria. *Soil Sci.* 24: 414–419.

(14) SEARS, O. H., AND CLARK, F. M. 1929 Non-reciprocal interchangeability of the Wood's clover and garden bean nodule bacteria. *Jour. Bact.* 17: 20.

(15) SIMON, J. 1914 Ueber die Verwandschaftsverhaltnisse der Leguminosen Wurzel-Bakterien. *Centbl. Bakt.* (II) 41: 470–480.

(16) STEVENS, J. W. 1923 Can all strains of a specific organism be recognized by agglutination? *Jour. Infect. Diseases* 33: 557–566.

(17) STEVENS, J. W. 1925 A study of various strains of Bacillus radicicola from nodules of alfalfa and sweet clover. *Soil Sci.* 20: 45–61.

(18) THORNTON, H. G. 1930 The early development of the root nodule of lucerne (*Medicago sativa*, L.) *Ann. Bot.* 44: 385–392.

(19) VOGEL, J., AND ZIPFEL, H. 1921 Beitrage zur Frage der Verwandtschaftverhaltnisse der Leguminosen-Knollchenbakterien und deren Artsbestimum ung mittels serologischer Untersuchsungsmethoden. *Centbl. Bakt.* (II) 54: 13.

(20) VOORHEES, J. H. 1915 Variation in soybean inoculation. *Jour. Amer. Soc. Agron.* 7: 139–140.

(21) WALKER, R. H. 1928 Physiological studies on the nitrogen fixing bacteria of the genus Rhizobium. Ia. Agr. Exp. Sta. Bul. 113.

(22) WHITING, A. L., FRED, E. B., AND HELZ, G. E. 1926 A study of the root-nodule bacteria of Wood's clover (*Dalea alopecuroides*). *Soil Sci.* 22: 467–471.

(23) WRIGHT, W. H. 1925 The nodule bacteria of soybeans: I. Bacteriology of strains; II. Nitrogen-fixation experiments. *Soil Sci.* 20: 95–140.

(24) ZIPFEL, H. 1912 Beitrage zur Morphologie und Biologie der Knollchenbakterien der Leguminosen. *Centbl. Bakt.* (II) 32: 97–137.

PLATE 1

EFFECT OF INOCULATION WITH VARIOUS SOYBEAN AND COWPEA CULTURES ON THE GROWTH OF SOYBEAN AND COWPEA PLANTS

FIG. 1. Efficiency of soybean and cowpea nodule bacteria in symbiosis with the cowpea plant.

Pot 1—uninoculated control
2—inoculated with soybean culture 6
3—inoculated with cowpea culture 11
4—inoculated with soybean culture 915
5—inoculated with soybean culture 1
6—inoculated with cowpea culture 10
7—inoculated with cowpea culture 2

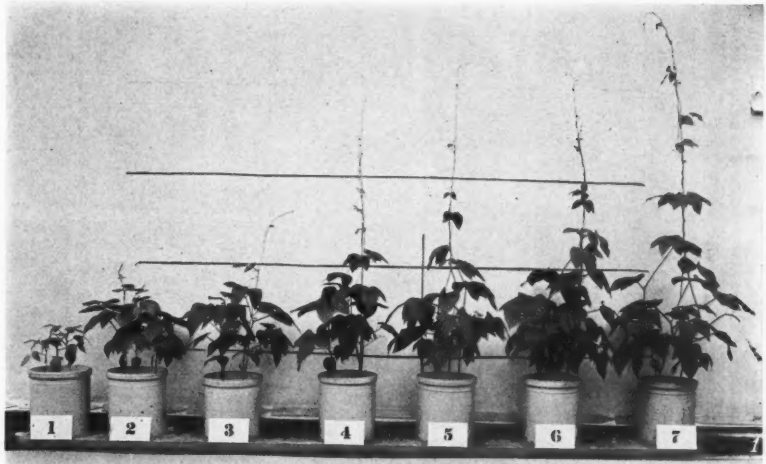
FIG. 2. Soybean plants showing variation in growth due to differences in nitrogen-fixing power of various cultures of soybean and cowpea nodule bacteria.

Pot 1—uninoculated control
2—inoculated with cowpea culture 28
3—inoculated with soybean culture 6
4—inoculated with soybean culture 13
5—inoculated with soybean culture 1
6—inoculated with soybean culture 915
7—inoculated with cowpea culture 28

FIG. 3. Variation in growth due to the effect of different cultures on the cowpea plant.

The pot elevated behind the field test in each case shows the result of the greenhouse portion of the experiment. As is stated in the text, the high temperatures in the greenhouse retarded the development of the pot plants during the last 3 weeks of the test.

Plat and Pot 1—uninoculated control
2—inoculated with soybean culture 5
3—inoculated with cowpea culture 9
4—inoculated with cowpea culture 31
5—inoculated with cowpea culture 600
6—inoculated with cowpea culture 23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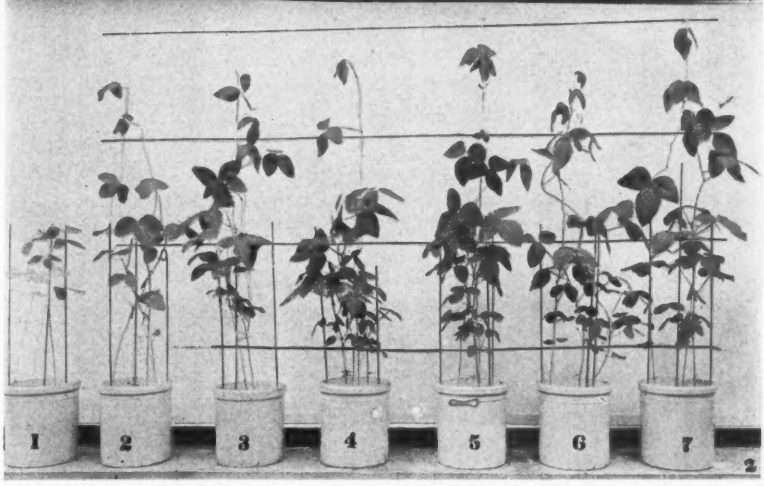
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PLATE 2

FIG. 1. The well-inoculated roots of cowpea plants in small plat 5 shown in plate 1, fig. 3.

FIG. 2. Morphological forms of cowpea nodule bacteria, culture 600 grown in asparagus-mannitol solution.

Photomicrograph 5—a dilute carbol fuchsin mount of a 48-hour culture—banded rods
6—nigrosin mounts of an 8-day culture—bacteroid stage
7—a nigrosin mount of a 17-day culture—coccoid stage

FIG. 1

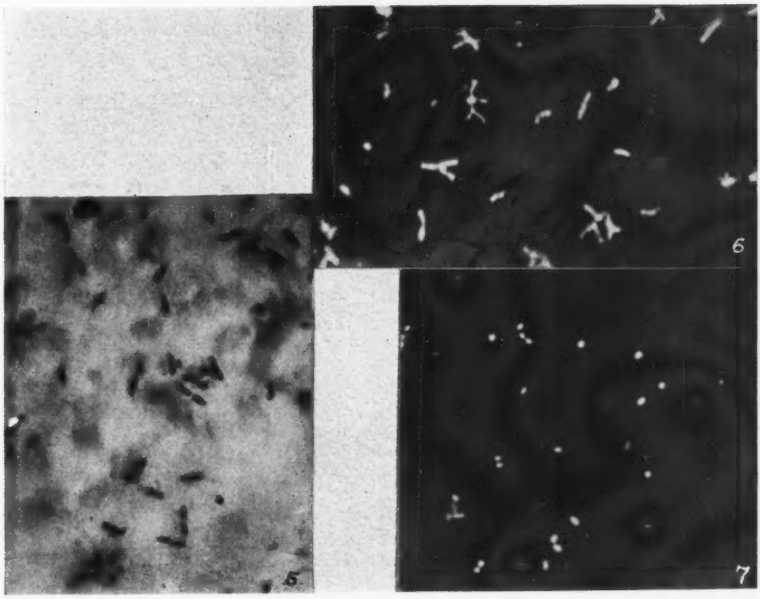

FIG. 2